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AMERICAN
JOURNAL OF BOTANY

VOL. III JANUARY, 1916 NO. 1

PHYSIOLOGICAL OBSERVATIONS ON ALKALOIDS, LATEX AND OXIDASES IN PAPAVER SOMNIFERUM*

RODNEY H. TRUE AND W. W. STOCKBERGER

In the summer seasons of 1902–4, while carrying on a series of investigations on the opium poppy, the writers made a number of observations on the occurrence and behavior of certain oxidizing enzymes present in this plant. These results, although verified by a number of repetitions, were not published at that time, it being then intended by the writers to broaden somewhat the scope of the investigation before bringing forward their results. However, the continuance of the work was subject to a number of contingencies and was not developed in the manner anticipated. Although much has happened since then that might concern the interpretation of the data then gathered, the facts themselves seem to point to certain broad conclusions which now as then are likely to interest the plant physiologist. Accordingly, it has seemed still in order to present these results with this explanation.

The plant material here used was grown by Mr. S. C. Hood, scientific assistant, from authentic seed at Burlington, Vermont, in the experimental grounds of the Vermont Agricultural Experiment Station in connection with co-operative work being carried on between that station and the Bureau of Plant Industry.

DISTRIBUTION OF OXIDASES AND OF LATEX IN THE PLANT

The oxidase was detected chiefly by means of the guaiac tincture test for oxidases of the laccase type used by Schönbein[1] and many

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* Published by permission of the Secretary of Agriculture.

[1] Schönbein, C. F. Ueber das Vorkommen des thätigen Sauerstoffs in organischen Materien. Journ. Prakt. Chem. **105**: 203–308. 1868.

[The **Journal** for December (**2**: 505–582) was issued Jan. 3, 1916]

others. The peroxidase reaction was studied chiefly by means of the guaiac tincture followed by hydrogen peroxide. The guaiac test was supplemented by the test with pyrogallol and sometimes with gallic acid.

The presence of an oxidase of the general type represented by the laccase of Bertrand and by the tobacco oxidase of Loew was easily demonstrated. This oxidase as found in the freshly expressed juice gave a reaction with guaiac tincture, pyrogallol and gallic acid. After precipitation with an excess of strong alcohol, the solution obtained on redissolving the precipitate in distilled water gave an intense reaction. Rough tests showed that in such solutions both the oxidase and the peroxidase reactions were inhibited by an exposure for four minutes to a temperature of 70° C. It appeared that this limit varied with the concentration and age of the enzyme solution. This inhibition of the reaction made it clear that the causes of the color changes lay in something easily modified by heat, not in any of the more stable substances shown to be capable of bringing about like color changes in the guaiac tincture. It having been seen in preliminary tests that the oxidase and peroxidase reactions in the different organs of the plant differed widely in intensity, systematic data were sought on this point.

In order to get evidence on the question of distribution from fresh materials, the reagents were taken to the field and applied to freshly cut surfaces of the growing plants. Here the order of intensity shown by the guaiac reaction agreed with that seen in the discoloration of the pulp. The most marked oxidase reaction was always seen in the more active younger parts of the plant. The fresh roots showed an almost complete lack of oxidase while the buds and petals were heavily loaded with it. The conclusion seemed justified that in this plant the intensity of the oxidase reaction increases from the base toward the summit of the plant. Similar tests for the peroxidase reaction showed clearly the presence in all parts of the plant of substances causing this reaction and no marked difference in intensity seemed to characterize any special part of the plant unless a greater activity was seen in the buds and flower parts.

The question immediately presented itself as to what particular tissues or substances contained the oxidases. When the guaiac tincture was applied to the cut surface of the growing plant, the drops of latex which instantly appear first gave the oxidase color reaction and remained much more intensely colored than the surrounding parts. When the latex was gathered by allowing the exuding drops to fall into a little distilled water an intense oxidase reaction was likewise seen. A study of the reaction on cut surfaces of leaves, stems and roots showed that the reaction was most intense where the latex was most abundant and that, indeed, as far as could be judged by this crude method, the latex content and oxidase reaction ran roughly parallel. The petals seemed to form a possible exception in giving an intense oxidase reaction while yielding little latex on wounding. However, the mass of tissue here is small and the very numerous small branches of the latex system may offer obstacles to the quick and abundant outflow of the latex such as would be strikingly seen on the cut surfaces of the more massive structures.

Further interesting light on the relation of the latex to the oxidase reaction came from a study of young poppy plants. Plants from 30 to 45 centimeters tall on which no flower buds had as yet appeared gave no clear oxidase reaction on cut surfaces, and the plant juices were watery rather than milky. As the plants developed the suspended matter giving to the latex its characteristic milky appearance increased and the oxidase reaction also appeared as already described. Whether this coincidence between the degree of milkiness of the latex and the intensity of the oxidase reaction has any special physiological significance can not now be stated. However, a number of wild plants having a milky juice were tested in the same way and a strong oxidase reaction appeared whenever the juice was treated with the guaiac tincture. The addition of H_2O_2 in these cases gave a very strong reaction for peroxidase. The following plants were tested: *Euphorbia maculata, Sonchus asper* and *Hieracium aurantiacum.*

The study of these reactions on fresh plants was supplemented by a laboratory examination of extracts prepared from different parts of the poppy plant. Normal poppy plants approaching maturity were carefully dug up, promptly and thoroughly cleaned and quickly cut into the following portions: Roots, lower stems, leaves, upper stems, capsules (immature), and flower buds. Each portion was quickly reduced to a fine pulp by use of a meat grinder, placed in a clean beaker similar in size and shape to the others used in the series and macerated over night in a volume of water proportional to the weight of the fresh pulp and sufficient to cover it. On the following morning the various macerations were found to have undergone a change of color in the surface layer. The root material showed but a slight change of color, a grayish tint being seen rather than the reddish-brown color characteristic of the others. The material from the stem portions was slightly reddish brown, with a distinctly more intense color toward the upper part of the plant. This intensity further increased in the leaf material. The reaction still more intense in the capsules was exceeded by the flower buds which gave a most intense color. The petals and stamens were also shown by separate tests to be most active. Tests with litmus paper showed that the extracts from the petals, lower stem and roots were neutral. All others showed a trace of acidity. In so far as this evidence went it seemed to indicate that the oxidase reaction was most abundant in the younger, growing parts of the plant.

The solutions of the different portions of the plant after being expressed from the pulp were treated with three volumes of commercial alcohol and briskly shaken. Precipitation was complete after several hours when the precipitate was filtered. This precipitate was dissolved in water as far as possible but a considerable insoluble residue always remained. The resulting solutions when tested for the oxidase reaction gave distinct though not strong oxidase reactions which in order of intensity was nearly the reverse of the order seen in macerated materials after standing over night in the beakers.

This interesting result seemed to indicate several possibilities. If it be assumed that the browning of the surface layer of the watery extract was caused by the precipitated substances responsible for the blue color in the guaiac tincture an apparent contradiction in the evidence seemed to exist here. That it may, however, be apparent only seemed to follow from further experiments.

In the course of the preparation of enzyme-containing precipitates the ground pulp was macerated in water over night in loosely covered beakers. In the morning a more or less deeply colored brownish layer was seen at the top of the material. Portions of the solution which were carefully drawn off by means of a pipette from this colored layer and from the uncolored portion near the bottom showed a marked difference in their activity toward the guaiac solution. Although the color of the superficial layer seemed to indicate that marked oxidative activities had taken place in the region of contact with the air, but a very faint oxidase reaction was seen when the reagents were employed. On the other hand, the uncolored portion from the bottom of the beaker gave a strong oxidase reaction. The cause of the disappearance of the oxidase reaction from the brown surface solution was next sought.

A very active oxidase solution was prepared by quickly grinding up buds in distilled water and filtering off the solid portions. This solution which immediately began to take on a brownish tinge on exposure to the air was quickly divided into two portions. One was put into a bottle which was completely filled, tightly stoppered and placed near the bottle through which air was being drawn. After about 20 hours the aerated solution was found to have taken on a dark-reddish brown color suggesting a coffee infusion indicating that an intense action had taken place under the influence of the greatly increased air contact. This solution showed an almost complete loss of the oxidase reaction when tested with guaiac tincture. The second portion preserved out of contact with the air and which showed no clear deepening of color during the interval gave a very strong oxidase reaction. Both solutions were neutral to litmus. It seemed to be indicated that either the oxidase had been exhausted during the reaction if still present or had been in some way inactivated.

It was thought possible that products of oxidation that without doubt had accumulated in the solution during aeration might in some way have inhibited the oxidase reaction. Accordingly an attempt was made to free at least partially the supposed enzyme from these products. The dark colored solution was treated with three volumes of commercial alcohol and the resulting bulky flocculent pale-colored precipitate filtered off after about two or three hours. The filtrate retained the brown color almost completely. The washed precipitate was thrown into a volume of distilled water equal to about half the volume of the original solution. As is usual with such precipitates a considerable part remained undissolved. The solution obtained carried a trace of color but not sufficient to obscure a definite oxidase reaction. Test with guaiac tincture, however, failed to give even minimum traces of such a reaction. Since by the same method active oxidases were regularly prepared from fresh material, it was clear that the process of isolation had not destroyed the enzymes. Although a considerable part of the products of enzyme action had without doubt been removed no return of oxidase activity was seen, a fact strengthening the suggestion that the enzyme was exhausted or inactivated through use. It is of course not clear to what degree the process of precipitation and re-solution separated the oxidase from the oxidation products. In so far as color may be accepted as an index, it seems probable that a very considerable degree of separation was effected.

Taking all evidence into account, the conclusion was strongly indicated that the enzyme was used up or inactivated during the course of the reaction. It is interesting to note in this connection the similar conclusions arrived at by Bunzel[2] in his recent and more exact studies.

In order to get further evidence on this point, a series of experiments was made with the juice of potato tubers. Freshly prepared aqueous extracts made in the same way as the poppy extracts gave active oxidase and peroxidase reactions. The solutions darkened very rapidly on standing and when tested after four hours gave no oxidase reaction. This solution was then treated with two volumes of strong alcohol, filtered after about an hour and the precipitate dissolved in distilled water. The resulting solution gave no oxidase reaction. The conclusion drawn from the poppy experiments seemed to be strengthened by the evidence gained from the work done on potatoes. This conclusion is hardly compatible with a catalytic explanation of oxidase action.

It is recalled that in the making of opium, the crude material from which morphine and several other alkaloids are obtained, the essential process consists in so scarring the full-grown but still green capsules as to cause the latex to run out onto the surface where in contact with the air it dries down from a thinly fluid milky juice to the dark brown, gummy substance known as opium.

Distribution of Alkaloids in the Plant

In view of the relations just discussed, it seemed desirable to ascertain in how far the distribution of the alkaloid, morphine, might show a relation to the distribution of latex and of the oxidase reaction. Accordingly, a number of full-grown plants of the black-seeded form of the opium poppy a meter or more high were brought in to the laboratory where they were cut up as quickly as possible into the following parts: Roots, lower stem, midstem, upper stem, leaves, flower buds, and capsules. The capsules were approximately full-grown but were still green and full of sap. Each of these portions was separately ground to a pulp and twice extracted with a hydro-alcoholic menstruum. The green weight was taken just before grinding and the pulp after extraction was expressed and again weighed. The amount of crude morphine was then determined for each extract and calculated with reference to the quantity of the plant material which had yielded it.

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[2] Bunzel, H. H. The measurement of the oxidase content of plant juices. Bulletin 238, Bureau of Plant Industry, U. S. Dept. Agric. 1912.

The results of the morphine determination are expressed in a ratio of morphine present to the same unit weight of plant material calculated for each of the plant samples. The relative yields of morphine were as follows:

Root	29.0	Lower stem	2.6	Midstem	1.3
Upper stem	2.2	Leaves	6.1	Buds	102.0
Capsules	42.0				

Some morphine was found in all parts of the plant. The roots in which there seemed to be but little latex yielded morphine in fair quantity, while in all parts of the stem the amount was almost negligible. However, the highest yield of morphine by far was found in the buds and capsules, both actively developing structures. Disregarding the root, the distribution of morphine in the plant seemed to conform fairly well to the distribution of oxidases and latex as previously noted.

The suggestion of a direct relation between the oxidases and morphine formation led to a series of observations on the latex itself. It was found that when the latex flowing from the freshly incised capsules was allowed to fall directly into strong alcohol the material failed to respond to the usual qualitative tests for alkaloids, while similar samples collected at the same time either in water or in a petri dish where it was allowed to dry gave all of the usual reactions with the common alkaloidal reagents. Fresh latex from the same plant was repeatedly collected both in alcohol and in water and tested with the result just described.

Conditions of Alkaloid Formation in the Plant

The results would seem to be explained on the assumption that morphine does not exist preformed either in the living plant or in the latex but develops in the latter when it is exposed to the action of the air or in the plant through oxidation changes as the tissues mature and die. Apparently the oxidase which occurs so abundantly in the latex may be credited with playing an important part in these oxidations. Morphine was absent in the solutions of the free latex only when oxygen was excluded or when the action of the oxidases was inhibited.

Further evidence on the part played by the air in the production of morphine was added as a result of an experiment in which fresh capsules of the poppy were dried in an atmosphere from which air (oxygen) was excluded.

Three uniform lots of fresh poppy capsules of normal growth were collected on the tenth day after the fall of the petals.

Lot I was spread out on a bench at a north window of the laboratory and allowed to dry by simple exposure to the air.

Lot II was dried out in an air bath oven at a temperature varying between 90 and 100° C.

Lot III was dried out in an atmosphere of CO_2. A Remington copper still filled with CO_2 was taken to the poppy field where selected capsules were cut off and immediately put into the still through a suitable opening. This opening which was near the top of the container was tightly stoppered except when opened to receive the capsules. When the desired quantity of capsules had been collected, the still was brought back to the laboratory and in large part submerged in a water bath in which from 8 o'clock a.m. to 5 p.m. the water had a temperature close to the boiling point. No heat was applied during the night. As soon as the still with its load of capsules was in position in this water bath connection was made with a cylinder of liquid CO_2. The gas passed first through a wash bottle containing sulphuric acid, thence through a glass tube dipping into a vessel of boiling water in which the gas was heated. From here it was carried to the bottom of the still where it diffused among the capsules. The excess CO_2 and the vapor from the drying capsules escaped through a small opening in the top of the still. A continual flow of gas was maintained day and night until the capsules were dry. When dry, the capsules were collapsed, brown in color, and very brittle.

The three lots of material were analyzed by Mr. W. O. Richtmann, at that time pharmacognostical expert in this bureau. Those dried in the open air at room temperature and in the air-bath oven were found to contain normal amounts of total alkaloids. The lot dried in the atmosphere of CO_2 contained no aklaloids at all.

An attempt was made the following year to repeat this experiment.

Mr. Hood collected and dried two lots of capsules, one in contact with the air, the other in an atmosphere of CO_2. Unfortunately, however, the experiment was hardly a repetition. Some of the capsules had begun to desiccate before the experiment was set up. An accident occurred to the container holding the lot drying in CO_2 with the result that the material was exposed for some time to the air. The experiment, however, was carried through. On analysis by Mr. Richtmann the air-dried material was found to contain 0.064 percent crude morphine, those dried in CO_2, 0.032 percent crude morphine calculated on dry weight at about 60° C. When the modifying conditions just described are taken into account, the results obtained seem to confirm those of the first experiment.

From the evidence at hand, we believe ourselves justified in tentatively advancing the conclusion that morphine as such does not exist in the poppy but is formed from a mother substance present in the latex through the action of oxidases using the oxygen of the air. It seems quite probable that the mother substance consists of a complex molecule which, under the action of atmospheric oxygen wielded by oxidases, is split along a fairly well determined cleavage line with the result that a rather constant N-containing product having the constitution of the alkaloid morphine arises. Should the reaction occur under somewhat different conditions, it seems possible that the lines of cleavage might shift somewhat, giving a different proportional quantity among the many alkaloids obtained from the poppy. When oxygen is absent and presumably oxidase action also, cleavage, if it takes place at all, may take place along quite different lines with the result that no morphine appears. That other alkaloids are affected as well as morphine is shown by the entire absence of an alkaloidal reaction in the material dried in CO_2. A certain kind of analogy between this situation and that seen in glucosides which are split up through the action of enyzmes is strongly suggested.

Inasmuch as physiological opinion concerning the significance of alkaloids to the plants producing them has tended strongly toward the view that they are waste products of plant metabolism, it seemed desirable to carry the investigation further. Obviously morphine itself can hardly represent to the poppy plant an accumulation of N-containing waste products.

It could hardly be taken for granted, however, that all alkaloids stand in a like relation to the plant. Accordingly, belladonna plants grown on the experimental farm of the Department at Arlington Farm, Va., were used as material for experimental study. This plant was chosen because of the absence of latex and because it is a fair representative of a large and much studied group of alkaloid-bearing plants. The roots, known to be rich in alkaloids, chiefly atropine, were dug in November, 1905, quickly and carefully washed, cut into transverse slices from 3 to 6 mm. thick and divided into two lots. It has been shown by Sievers[3] that there is a wide range of individual variation in alkaloidal content in belladonna. The chance for error from this source was reduced by dividing the slices of each root equally between the two lots.

One lot of 450 grams fresh weight was placed in a glass jar the mouth of which was closed except for holes to permit the escape of excess gas and water vapor. CO_2 was led from a tank through sulphuric acid, thence through a copper coil immersed in boiling water to give the gas a greater water-absorbing capacity and thence into the bottom of the jar containing the sliced roots. The jar itself stood in a water bath kept at boiling temperature from 9 a.m. to 4:30 p.m. while the gas flow was maintained throughout the day. The material was dry and hard on December 2.

A second lot was placed in a similar jar likewise heated in a water bath, but provided with a supply of air instead of CO_2. Drying was finished on November 29 when the material was hard and dry and had a pronounced odor of brown sugar.

Duplicate analyses of the powdered material made by Mr. W. O. Richtmann showed the following result:

TOTAL ALKALOIDS IN BELLADONNA ROOTS

Treatment	Sample	Total Alkaloids, Percent
Dried in CO_2 current	*A*	0.665
	B	0.665
Dried in air current	*A*	0.642
	B	0.625

These data show clearly that the alkaloidal yield of belladonna root is not dependent on the action of oxygen and, therefore, is governed by physiological conditions quite different from those governing the formation of morphine. The application of guaiac solution to the freshly cut root produced a very strong oxidase reaction, apparently most intense in the cortical regions. The addition of hydrogen peroxide did not markedly intensify the color.

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[3] Sievers, A. F. Individual variation in the alkaloidal content of belladonna plants. Journ. Agr. Research **1**: 129–146. 1913.

As far as the evidence here submitted goes, the belladonna alkaloids exist ready formed in the plant perhaps as accumulated waste products.

SUMMARY

In conclusion, the results here reported may be briefly summarized:

1. It appears from work done on the opium poppy, *Papaver somniferum*, that the oxidase reaction is most active in the upper parts of the plant, especially in the floral structures, capsules and actively growing parts. The peroxidase reaction shows less variation in its intensity in the different parts of the plant.
2. The intensity of the oxidase reaction roughly parallels the distribution of the latex which in itself is most active.
3. The oxidase seems to be either "used up" or otherwise inactivated during the course of its action. A like exhaustion or inactivation of the supposed enzyme was seen in the case of the oxidase of the Irish potato. These observations would indicate that perhaps the oxidase reaction is not due to a catalyzing agent, therefore is not due to an enzyme.
4. With exception of the root, the intensity of the oxidase reaction runs roughly parallel with the alkaloidal content. In the root, the alkaloid content is relatively higher than the intensity of the oxidase reaction.
5. Alkaloids seem not to exist as such in the poppy plant but to appear as products of the action of the oxidases on constituents present in the latex reacting in the presence of oxygen.
6. The alkaloids of *Atropa belladonna* differ from those of the poppy in that they are found to exist in structures dried without contact with free oxygen and seem to exist ready formed in the plant.

OFFICE OF DRUG PLANT, POISONOUS PLANT,
PHYSIOLOGICAL AND FERMENTATION INVESTIGATIONS,
BUREAU OF PLANT INDUSTRY, U. S. DEPARTMENT OF AGRICULTURE.

NOTES ON THE ANATOMY OF PERIDERMIUM GALLS. I

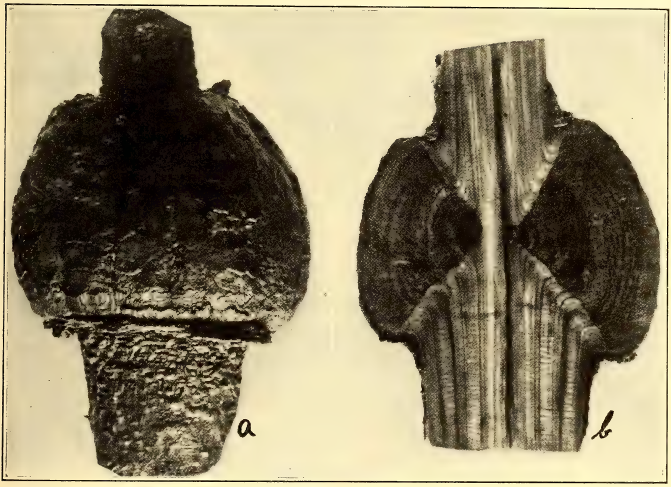
ALBAN STEWART

Peridermium (Aecidium) *cerebrum* Pk. on *Pinus Banksiana* Lamb.

Peridermium cerebrum parasitizes fourteen species of North American pines according to Arthur and Kern (3, pp. 134–135). Owing to the wide range of these species the distribution of the fungus is also wide, occurring nearly throughout the United States, southward to central Mexico, and northward along the mountains to southern Alaska according to the above named authors. The jack pine, *Pinus Banksiana*, is one of the species infected by this parasite, large woody galls resulting on the branches from its attack. A number of these galls came into my possession some time ago, and as a preliminary examination revealed some rather interesting anatomical peculiarities, I thought the material to be worthy of a more careful study. The results of this are given in the following pages.

The galls examined vary considerably in size and age but they all occur on rather young branches. The largest one examined has a cross diameter of 4.7 cm. and the smallest one a similar diameter of about 1 cm. They usually occur singly but two may sometimes be closely associated and later joined together. The outer surface is much roughened due to the scaling off of the outer bark (text-fig. 1*a*), a character which is seemingly more pronounced when spores are being discharged than at other times of the year.

The woody portion of the gall is sharply set off from the normal wood above, below, and inside by the difference in color. The normal wood is light in color, while that of the gall is distinctly brown, probably due to accumulations of resin and other substances in it. This brown coloration is usually more pronounced towards the center, sometimes the outer layers being free from it. In such cases alburnum and duramen portions are well differentiated. Without exception all galls examined show a deep coloration into the first ring of growth at some point or points in the circumference, and tissue abnormalities accompany it. Evidently the fungus was present when this wood was formed and the brown color is not due, at least not entirely, to an infiltration of substances from the layers of wood further out. The galls examined in this respect were of various sizes, some large, others small. More especially were the rather small ones on larger branches examined as these seemed most likely to show an exception if such were true. None of them did, however, so it seems likely that the infection usually if not always takes place during the first year's growth of the branch which bears the gall. This, however, is not true for all species of Peridermium, as Hartig (8, p. 173) describes an instance in which a pine was attacked by *P. Pini* in the fifteenth year of its growth. According to Anderson (2, p. 311) *P. elatinum* seldom attacks branches of *Abies balsamea* over five years old, while *Abies pectinata*, according to de Bary (4, p. 257), is attacked both on young and old parts, and a case is reported where a stem a foot thick and having 60–70 rings of growth was infected.



TEXT-FIGURE 1. Gall of *Peridermium cerebrum* on *Pinus Banksiana*. *a*, external view of the gall, *b*, median longitudinal section of gall. (Both figures about natural size.)

A median longitudinal section through one of the larger galls is shown in text-figure 1*b*. This gall is probably seven years old, as there are that many faintly marked rings of growth in it which correspond with the number of rings in the normal part on the stem just below it. Cross sections of other galls, which were examined microscopically, also show a correspondence in a number of rings both in the gall and in the stem close by. One side of the stem where the gall is located (see text-figure 1*b*) has not as yet become changed at the center, showing that the fungus may spread very slowly in a peripheral direction. The two sides of the stem in this figure show a difference in time of infection, as the wood on the right side near the center is abnormal nearly to the pith, while on the left side there are nearly two rings of normal wood next the pith. The fungus evidently spreads quite as slowly vertically as it does horizontally. The lighter portion in the center of the gall (text-figure 1*b*) is composed almost entirely of normal wood. These zones become broader towards the upper and lower sides so that the gall encloses two more or less cone-like masses of normal wood the apices of which meet near the center. Furthermore it can be seen in this figure that the bark[1] is not appreciably thicker than the bark of the normal stem above and below. The outer portions of this are shed rapidly by the formation of periderm so that nothing remains but the inner phloem and rays. The phloem portion is composed mostly of large cells containing resin and other substances. According to Hartmann (9, p. 32) the bark may become more than three times as thick as normal on branches of the white fir infected with *Peridermium elatinum;* according to de Bary (4, p. 258) however, it may be as much as ten times as thick as normal.

The first striking abnormality to appear in going from the normal wood to that of the gill is a sudden bending outward of the tracheids. The pitting in the walls of these differs rather strikingly from normal in places. According to Penhallow (13, p. 321) the bordered pits in the radial walls of the tracheids in this species of pine are arranged in one row, uniseriate, sometimes in pairs or distinctly two rowed. Specimens of normal wood examined show that the biseriate arrangement rarely occurs. In the wood of the gall the size and arrangement of the pits may differ greatly from normal. The usual arrangement is uniseriate, but two rows of pits often occur and rarely three rows. Where the arrangement is biseriate the pits are usually opposite, but instances are common where there is a strong suggestion of alternate pitting. Illustrating of this tendency appears in plate I, figures 3 and 4, which show about average conditions in this respect. A stronger tendency towards an alternate arrangement occurs sometimes. Rarely both an opposite and an alternate arrangement of pits takes place in the same tracheid wall and occasionally there are transitional stages between these two methods. Where the pits alternate they are sometimes slightly flattened by mutual contact. (See plate I, figure 3). Thomson (16, p. 17) mentions alternate pitting in the cone axis and early wood of the Abietineae and that the pits are sometimes flattened by contact. He also shows on his plate IV, figure 36*c*, a tracheid from the young root wood of *Larix americana* in which there is a suggestion of both opposite and alternate arrangement of pits, a condition which is not strikingly different from what sometimes occurs in the gall under consideration. A slight tendency towards an alternate arrangement of pits is in reality not an uncommon feature in several species of pines. It occurs to a greater or less extent in *P. echinata*, *P. lambertiana*, *P. palustris*, and *P. strobus*. Occurring as it does in several species of pines it is very likely that careful search would reveal it in others. It probably has no great significance in Pinus.

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[1] See Barnes, C. R. "What is Bark?" Bot. Gaz. **22**: 237. 1896.

There is a great difference in the size of the pits in the tracheids. They are usually about normal in this respect, but sometimes there are bordered pits present which are very much smaller, often about one fourth the usual size. Large and small pits may occur closely associated in the same tracheid wall. The pits may be scattered or closely arranged (figure 4).

According to Gerry (6, p. 122) the pines and other conifers with abietineous affinities always have bars of Sanio present in the walls of the tracheids, while in the Araucarineae they never occur. Jeffrey (10, 544–545), however, has subsequently found them to be present in certain regions of *Araucaria Bidwellii* and *A. imbricata*. By staining with Haidenhain's haematoxylin and safranin according to the method described by Jeffrey (l.c., p. 547), these bars can be easily seen in some of the tracheids of the gall but they fail to appear in others. They may stand out sharply in one while in another adjacent tracheid they can not be seen, or, they may appear in one part of the wall and not in another. Very faint traces of them occur at times. As so much depends on the methods of staining in order to see these bars, it is hardly safe to say positively that they are absent at times from the tracheids of this gall. If they are invariably present I have not always been able to see them. They are probably sometimes absent. Thomson (16, p. 22, pl. 4, fig. 36*a*) has found a somewhat similar condition, as he states that "where the pits are multiseriate, as in young root wood, '*Larix americana*' there may not be a trace of the bar of Sanio."

The walls vary in thickness, individual tracheids showing differences in this respect. This condition is very noticeable in longitudinal sections, but it is less marked in cross sections (see plate I, figure 1). The walls are often sinuous (figure 4), causing wide and narrow places in the same tracheid. Where this condition is pronounced inflations in the walls may result from it. Blunt end walls are common. The length of the tracheids varies greatly and as a whole they are shorter than in normal wood. Cells which are isodiametric or nearly so occur commonly, which show their tracheary character in the pitting. Wörnle (18, p. 132) has described similar cells near the broad rays in juniper stems infected with *Gymnosporangium uniperinum*. The formation of these short tracheids possibly comes about by the cross division of cambium daughter cells. Such is the case in traumatic wood according to de Vries (5, p. 21).

Cells occasionally occur which have the character of both tracheids and parenchyma cells as the pits in one part of the wall are bordered and in another part plain. The plain pits occur in such places as to leave no doubt about their true character. They were not mistaken for one-sided bordered pits as they do not occur in opposite rays. Mäule (11, p. 5, plate II, figure 1) describes and figures cells somewhat similar to these in the traumatic wood of *Abies cephalonica*, Thompson (15, p. 336) describes transitional cells which are normally present in the rays of *Abies homolepis*, and Groom and Rushton (7, p. 474, figure 16) mention simple pit-like structures between the bordered pits in *Pinus excelsa*, a species from the Himalayas. True wood parenchyma cells also occur in this gall, which have nuclei and other protoplasmic contents. Such cells do not occur normally in the pines according to Penhallow (13, p. 112). They appear to be more numerous among the tracheids which first bend outward from the normal wood into that of the gall, but this may be due to the fact that there is less resin in these parts so that they can be more readily seen there, and their true character recognized, than deeper in the gall.

Cross sections cut through the center of the gall usually show that the arrangement of the tracheids is not strikingly different from normal (see plate I, figure 1). Disturbance in the arrangement does occur sometimes in which the tracheids are usually turned over to right and left tangentially, probably indicating regions of ball-formation of tracheids. Similar conditions occur in juniper stems infected with *Gymnosporangium juniperinum* according to Wörnle (18, p. 83). The width of luminae and thickness of the walls of the tracheids does not differ greatly from that of normal cross sections except in the summer wood where the walls are much thinner than in normal summer wood (see plate I, figure 1). This is quite different from the wood of *Abies balsamea* infected with *Peridermium elatinum*, as Anderson (2, p. 337) has reported thicker walled tracheids than normal. There are but few layers of summer tracheids formed, and it is due to this fact that the rings of growth are sometimes not well defined. A similar condition may result in juniper stems infected with *Gymnosporangium juniperinum* according to Wörnle (l.c.). If cross sections are cut above or below the center of the gall they will usually show a structure between cross and tangential. This peculiar condition is due to the fact that the tracheids pursue a more or less horizontal course for some distance after they bend outward into the gall from the normal wood.

In radial sections the course of the tracheids follows in a general way the outer contour of the gall (text-figure 1*b*). There are some exceptions, however, as a nearly true transverse structure may occur as well as wavy structure. In tangential sections the normal course of the tracheids is often very much disturbed, resulting in wavy structure and balled or whorled arrangement. Large whorls, very complex in structure, sometimes occur in such sections (plate I, figure 2). According to Mäule (11, p. 10) these seldom appear in other than tangential sections of traumatic wood. I have found this to be true in this gall and in the gall of *Andricus punctatus* on the black oak, see Stewart (17, p. 541). Small tracheid whorls occur in traumatic wood of the jack pine but I have failed so far to find any which approach in any way the size of the one shown in figure 2. Large structures of this kind do sometimes occur in traumatic pine wood, however, as Mäule (l.c., p. 12) reports them from *Pinus Pumilio*. U- and Y-shaped tracheids are common in regions of extreme distortion in the gall.

In addition to the fusiform rays, which are low and scattered in the normal wood, three sorts of rays occur in the wood of the jack pine. According to Penhallow (13, p. 321) there are: "(1) low rays composed of oblong narrowly oval thin-walled parenchyma cells with narrowly oblong, terminal tracheids; (2) low rays of similar composition, but the parenchyma cells much thinner walled; (3) the highest rays composed chiefly of narrow tracheids with a few broader, oblong parenchyma cells interspersed." I have found it difficult to distinguish between groups 1 and 2, but the rays of group 3 are readily distinguished, and from the single specimen examined, I find that they are in general 6–10 cells high. Both low and high rays can be seen in tangential sections of the gall, the higher ones being the more common. These usually do not exceed the normal rays of group 3 in number of cells in height, but where exceptions occur the increase is largely due to the addition of more rows of parenchyma cells. The individual cells composing the rays are usually larger than normal.

With the exception of fusiform rays, Penhallow (13, p. 94) states that the rays are always uniseriate in Abies, Picea, and Pinus. It is a common thing to find rays in tangential sections of this gall which are two cells wide in part (plate I, figure 5) and occasionally a ray which is three cells wide. It is evident that these are not fusiform rays, which occur more abundantly in the gall than in normal wood, as they do not partake of the character of such rays. A similar condition of ray structure also occurs in traumatic wood of this species of pine (plate I, figure 6).

A sporadic tendency to a slight broadening of the rays occurs in a few of the living conifers according to Penhallow (13, p. 94). It may be met with in species of Pseudotsuga, Cupressus, Juniperus, Sequoia, Araucaria, and Larix. In Libocedrus the tendency is more pronounced. A significant broadening of the rays may take place according to Mäule (11, pl. 2, figure 8), in traumatic wood of *Abies cephalonica*, a genus in which such does not occur normally. Wörnle (18, p. 131, figure 8) has also recorded a marked broadening of the rays in stems of Juniperus infected with *Gymnosporangium juniperinum*, ray-like structures figured by this author being nearly as broad tangentially as they are high in some instances.

Certain authors have recently given a phylogenetic significance to the broadening of the rays in traumatic wood of some of the angiosperms. I believe that it is pretty generally accepted that the uniseriate ray is primitive in the Coniferae. If this conception is correct the broadening of the rays in its members, due to traumatic and other stimuli, can not be considered of phylogenetic importance. They may be formed to supply some need of the plant, and if such is true they are probably physiological rather than phylogenetic in their significance. Taken as a whole we know comparatively little about the structure of pathological plant tissue, and until more is known it is hardly safe to base any very strong conclusions on it phylogenetically.

The rays are more numerous in the gall than in normal wood, a given area of tangential section of the gall having approximately twice as many rays present as the same area of normal wood. The proportion of ray tissue is greater than this, as the cells are larger as a rule and more numerous in the rays of the gall. It might be well to mention in this connection that Anderson (2, p. 337) found that there were often twice as many rays present in parts of *Abies balsamea* infected with *Peridermium elatinum* as there were in normal parts.

The ray tracheids present some interesting peculiarities. In the normal wood of the jack pine these are strongly reticulated, in the gall the reticulations are often very much reduced or absent entirely. The ray tracheids thus combine characters of both hard and soft pines. Tracheids sometimes border the rays (center of plate I, figure 4) which are similar in many respects to the peculiar tracheids figured by Thompson (14) from the rays of the traumatic stem wood, and the normal root wood of *Pinus resinosa*.

Resin canals are few and widely scattered in the older wood of the jack pine but are more numerous in the younger wood near the pith. Close associations of canals occur but seldom in either the young or old wood. In cross sections of galls the canals may be as much as three times as numerous as in the normal wood, varying somewhat in different individuals. They are often arranged in concentric rings, so closely associated in places that only the rays intervene (plate I, figure 1). Anderson (1, p. 472) found a similar arrangement in parts of Abies infected with *Peridermium elatinum*. The canals in this gall have a lining of living nucleated cells and are evidently not merely resin passages such as are described by Mäule (11, p. 18) from the traumatic wood of certain conifers. A great increase over the normal number of resin canals sometimes takes place in traumatic pine wood. According to Thomson (16, p. 38) there may be 7–8 times as many as normal in the traumatic wood of *Pinus resinosa*.

An interesting thing in connection with the distribution of resin canals in this gall is the fact that there is no increase in the number in the uninfected parts above the gall. Anderson (1, p. 473) states that the parts of *Pinus Strobus* above infections of *Agaricus meleus* contain more resin canals than do normal branches. He also reports the presence of resin canals in the branches of Abies which are above infections of *Peridermium elatinum*. A similar condition is mentioned by Mer (12, p. 367) in Abies, above infections of *Phoma abietinae*.

We have in this instance a rather striking correlation between the effects produced by two species of the same fungus. In one case the fungus is unable to exert an influence on the structure of the host beyond where it is actually present in the tissues of the same, in the other case it is far reaching in its effects, and is not only able to modify the tissue of the host some distance from the parts infected, but is also able to stimulate the productions of organs that are not normally present there.

SUMMARY

The more important facts presented in this article are briefly summarized below:

1. Both an alternate and an opposite arrangement of bordered pits in the radial walls of the tracheids.
2. An unequal thickening of the walls and luminae of the tracheids.
3. Very short tracheids with blunt end walls, which resemble parenchyma cells except in the pitting.
4. Cells which are transitional between tracheids and parenchyma cells in the pitting.
5. The presence of true wood parenchyma cells.
6. A small production of thin-walled summer tracheids.
7. A probable absence of bars of Sanio from many of the tracheids.
8. An increase in the number of rays in the gall wood.
9. A tendency towards the production of multiseriate rays.
10. Ray tracheids which are transitional between those of both hard and soft pines.
11. The presence of a balled or whorled arrangement of tracheids in tangential section.
12. A great increase in the number of resin canals in the gall wood but no such increase in the uninfected wood close by. The examination of this gall has revealed so many points of anatomical interest that a further study of this subject seems to be worth while.

On this account the author expects from time to time to issue other papers on the anatomy of Peridermium galls on pines and other conifers.

METHODS

In preparing material for sectioning it was first treated with strong hydrofluoric acid for several weeks to soften the tissue. The sections were stained with Heidenhain's haematoxylin and safranin by the usual methods employed in staining wood sections.

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EXPLANATION OF PLATE I

All figures are from the jack pine, *Pinus Banksiana.*

FIG. 1. Cross section through a portion of a gall showing abnormally produced resin canals and thin-walled summer tracheids. × 65.

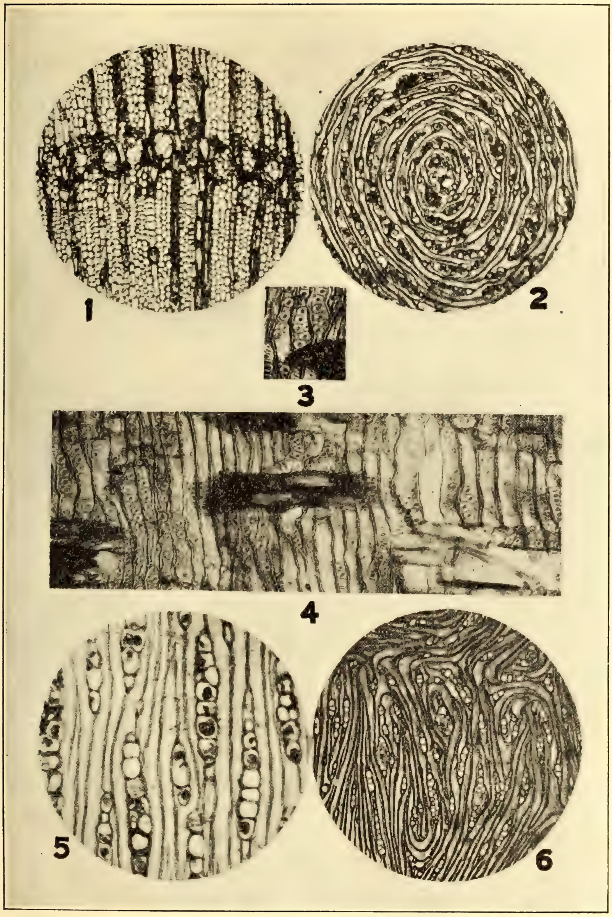
FIG. 2. Tracheid whorl or "ball-formation" from tangential section of gall. × 65.

FIG. 3. Portion of a radial section through gall showing alternate arrangement of pits. × 125.

FIG. 4. Portion of a radial section through gall showing irregularities in walls of tracheids, peculiar ray tracheids, and alternate pitting. × 125.

FIG. 5. Portion of a tangential section through gall showing a tendency towards a broadening of the rays. × 125.

FIG. 6. Tangential section of traumatic wood. × 65.



STEWART: ANATOMY OF PREIDERMIUM GALLS.

THE CLIMATIC DISTRIBUTION OF CERTAIN TYPES OF ANGIOSPERM LEAVES[1]

Irving W. Bailey and Edmund W. Sinnott

The instability of the gross or superficial characters of leaves has been emphasized by many taxonomists, and by a number of morphologists who have desired to bring into the limelight the conservatism of internal structures. Indeed, the prevailing opinion among botanists seems to be that foliar characters, except among small groups of closely related species, are so unreliable as to be of little value in the study of relationship and phylogeny. The warmest supporters of the opposite view, as might naturally be expected, are to be found among those paleontologists whose attention has been focused upon the identification of leaf impressions.

It occurred to the writers that a careful study of the distribution of various types of Angiosperm leaves in the principal phytogeographical regions of the earth might throw some light upon the question of the conservatism of foliar characters and their modification by environmental factors.

Fortunately, the task of tracing the distribution of foliar structures, particularly of the more conspicuous external ones, is facilitated by the fact that there are now available numerous published floras and large herbaria where descriptions of the leaves of plants from various parts of the world can be obtained. It is to be regretted, however, that so many taxonomists have made their floras and collections representative of political rather than of phytogeographical areas, and that ecological notes usually are meager or entirely absent.

In the following pages are summarized the results of an investigation upon the leaf form of Dicotyledons, undertaken in an endeavor to secure more specific information in regard to the distribution of leaves and leaflets with entire and non-entire (*Crenulate*, *crenate*, *serrulate*, *serrate*, *denticulate*, *dentate*, *lobed*, *incised*, etc.) margins.

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[1] Investigations upon the phylogeny of the Angiosperms, No. 6.

Table I[2] presents an analysis of the Dicotyledonous floras of various regions of the frigid, temperate, and tropical zones. The first column of figures on the left gives the total number of Dicotyledons, exclusive of aquatic, parasitic, and leafless forms, in the flora of each region. The three succeeding columns record the percentages of trees, shrubs (all ligneous forms except arborescent species) and herbs that occur in these floras; and in the last three columns are tabulated the percentages of the trees, of the shrubs, and of the herbs that have leaves or leaflets with entire margins.

TABLE I—*Group 1*

	Total Species	% *Trees*	% *Shrubs*	% *Herbs*	% Entire Trees	% Entire Shrubs	% Entire Herbs
Ellesmereland	68	0	9	91		100	48
N. E. Siberia	117	0	14	86		65	52

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[2] Based upon analyses of the following floras:

North America: The Vascular Plants in the Flora of Ellesmereland, Simmons; An Illustrated Flora of the Northern United States, Canada, and the British Possessions, Britton and Brown; New Manual of Botany of the Central Rocky Mountains, Coulter and Nelson; Flora of Los Angeles and Vicinity, Abrams; Flora of the Southeastern United States, Small; Flora of the Florida Keys, Small; Flora Nicaragüense, Goyena; Flora of the British West Indian Islands, Grisebach.

South America: Flora Braziliensis, Martius and others; Historia Fisica y Politica de Chili, Botanica, Gay; Reports of the Princeton University Expeditions to Patagonia, 1896–1899, Botany, Macloskie.

Europe: English Botany, Sowerby and others; Flora des Nordostdeutschen Flachlandes, Ascherson and Graebner; Flora Rossica, Ledebour; Flora Analitica D'Italia, Fiori and Paoletti; Herbier de la Flore Française, Cusin and Ausberque; Compendio de la Flora Española, Lazaro e Ibiza.

Asia: Flora Rossica, Ledebour; Flora Orientalis, Boissier; Flora Simlensis, Collett; Flora of the Upper Gangetic Plain and of the adjacent Siwalik and Sub-Himalayan Tracts, Duthie; Flora of the Presidency of Bombay, Cooke; A Handbook of the Flora of Ceylon, Trimen; Materials for a Flora of the Malayan Peninsula, King and Gamble; Flora Indiae Batavae, Miquel; Flora Hongkongensis, Bentham; Flora of Manila, Merrill; Woody Dicotyledonous flora of east central China, herbarium of the Arnold Arboretum.

Africa: Manual of the Flora of Egypt, Muschler; Flora of Tropical Africa, Oliver, Thiselton-Dyer and others; Flora Capensis, Harvey and others; Flora of Mauritius and the Seychelles, Baker.

Australasia: Flora Australiensis, Bentham; Manual of the New Zealand Flora, Cheeseman.

Oceana: Flora of the Hawaiian Islands, Hillebrand.

TABLE I—*Group 2 and Group 3*

	Total Species	% *Trees*	% *Shrubs*	% *Herbs*	% Entire Trees	% Entire Shrubs	% Entire Herbs
E. C. North America	1,504	7	14	79	10	37	42
Rocky Mountains	1,413	1	10	89	0	40	52
N. Russia	356	2	14	84	0	35	42
C. Russia	1,225	3	10	87	0	36	42
S. Russia	2,319	4	9	87	8	52	44
England	1,273	2	11	87	3	37	35
N. E. Germany	1,166	2	12	86	0	29	45
France	3,924	2	9	89	4	53	48
S. E. Siberia	458	2	10	88	0	45	44
W. Siberia	1,085	3	10	87	0	57	45
Kamtschatka	301	1	17	82	0	35	33
	15,024				2	41	43
S. E. United States	4,451	6	18	76	36	54	48
Los Angeles	831	3	16	81	18	58	45
Chili	2,409	4	30	66	46	55	46
Patagonia	1,488	2	28	70	28	57	47
Spain	4,103	1	18	81	13	59	48
Italy	3,069	2	10	88	18	56	40
E. C. China	2,015				38	52	
S. New Zealand	713	8	33	59	53	68	43
Simla (mts.)	989	13	25	62	53	60	38
	20,068				34	58	44

Among woody plants, leaves and leaflets with entire margins are overwhelmingly predominant in tropical and subtropical environments. This is very nearly as pronounced among shrubs as it is among trees. In cold-temperate regions, on the other hand, trees with entire leaves and leaflets are extremely infrequent, and the leaf-margins of shrubs are on the average more than half non-entire. Warm-temperate regions are intermediate between tropical and cold-temperate ones, and the shrubs of frigid habitats resemble those of the tropics in having very high percentages of entire margins.

Among herbaceous plants, higher percentages of leaves and leaflets with entire margins occur in tropical and subtropical regions, but the contrast between the frigid, temperate, and tropical zones is less well marked than among trees and shrubs.

The somewhat paradoxical behavior of the leaves and leaflets of arborescent, as compared with herbaceous Dicotyledons, raises the question as to whether the entire and non-entire types of leaf-margins are determined by environmental influences, or whether they are

TABLE I—*Group 4*

	Total Species	% *Trees*	% *Shrubs*	% *Herbs*	% Entire Trees	% Entire Shrubs	% Entire Herbs
Florida Keys	473	8	37	55	84	83	64
Nicaragua	1,509	17	39	44	86	71	48
West Indies	2,209	19	52	29	88	71	61
Brazil	10,468	21	62	17	87	76	56
Hongkong	699	16	43	41	73	71	58
Flora Orientalis	9,771	4	13	83	71	72	53
Upper Gangetic Plain	1,084	15	31	54	73	71	45
Malay States	3,252	41	42	17	90	82	64
Ceylon	1,752	20	44	36	84	78	54
Manila	333	30	38	32	83	80	67
East Indies	6,389	30	45	25	81	75	49
Hawaii	521	26	50	24	78	61	45
Queensland	1,734	22	45	33	81	82	63
W. Australia	2,543	2	73	25	73	83	71
New South Wales	1,780	13	54	33	75	84	54
Victoria	1,152	6	50	44	87	84	52
Tasmania	662	4	48	48	82	77	45
Egypt	1,239	2	16	82	73	78	49
C. E. Africa	2,837	6	36	58	75	74	54
C. W. Africa	2,656	17	46	37	85	80	56
S. W. Africa	2,400	11	43	46	84	82	62
S. E. Africa	2,653	8	42	50	80	78	56
Southern Africa	7,783	2	55	43	73	74	53
Mauritius-Seychelles	545	17	49	34	88	84	65
	66,444				81	77	56

form-variations of little functional significance that are held on by heredity?

In the first place, the possibility suggests itself that the entire-leaved plants in temperate regions and the non-entire-leaved forms in tropical regions may occur in somewhat different environments from those occupied by the prevailing types of arborescent vegetation. A detailed examination of the regions and percentages given in Table I affords some evidence that seems to point in this direction. All the regions recorded are heterogeneous in that they contain in most cases more than one of even the principal plant formations. In group 2, those regions, Rocky Mountains, south Russia, France, Siberia, etc., which have larger areas of arid or physiologically dry environments, have higher percentages of entire-leaved shrubs. On the other hand, those regions of the tropics that have more extensive equable environments or moist cool uplands have higher percentages of herbs with non-entire leaves and leaflets.

The influence of uplands, in increasing the number of non-entire types in tropical and subtropical regions, is also shown in the following table. It is in marked contrast to the effects of high alpine or cold, dry, upland environments in temperate regions.

TABLE II—*Tropical or Sub-tropical*

	Lowlands (% Entire)			Uplands (% Entire)		
	Trees, Percent	Shrubs, Percent	Herbs, Percent	Trees, Percent	Shrubs, Percent	Herbs, Percent
Hawaii	84	71	67	70	50	24
Ceylon	88	81	61	72	75	34
Temperate						
S. New Zealand	53	64	37	100	76	50

In view of these facts, it is desirable to compare a lowland-tropical flora and a uniformly mesophytic cold-temperate one. The next table gives an analysis of two such floras. The mesophytic cold-temperate flora was reconstructed from that of east central North America (east of the 95th meridian and between the 40th and 50th parallels of latitude) by eliminating all extremely microphyllous forms, xerophytes, and plants growing in dry habitats. The lowland-tropical flora was made from that of Brazil, by including the plants of the lowlands of the Amazon valley and excluding those from the uplands of the southern, central, and eastern provinces.

TABLE III

	Entire %		
	Trees, percent.	Shrubs, percent.	Herbs, percent.
Mesophytic Cold-temperate (E. C. N. A.)	10	14	23
Lowland-tropical. (Brazil)	90	87	62

Of course, it should be kept in mind that prevailing climatic influences are not absolutely constant throughout these extensive areas. Furthermore, it is hardly to be expected that in any such arbitrarily selected region of North America there should be an abrupt transition from cold-temperate to warm-temperate or to arctic conditions.

It is significant, therefore, that those arborescent species which constitute the 10 per cent of entire-leaved plants in the mesophytic cold-temperate flora are all southern, warm-temperate types, most of which are at one extremity of their range, north of their region of optimum development. They are:

Quercus phellos L.	*Magnolia acuminata* L.
Q. imbricaria Michx.	*M. virginiana* L.
Cercis canadensis L.	*Gymnocladus dioica* (L.) Koch.
Nyssa sylvatica Marsh.	*Diospyros virginiana* L.

Among the entire-leaved shrubs and herbs there are many frigid and warm-temperate types that are largely confined to the northern or southern portions of the region respectively.

In considering the percentages of non-entire leaves in the lowland-tropical flora, it is important to note that the serrations, dentations, etc., of the non-entire leaves, particularly of trees and shrubs, are very frequently vestigial or rudimentary. In fact, not only are the non-entire types of margins less numerous than they are in the uplands of southern and southeastern Brazil, but they differ from them in showing obvious signs of reduction. Similar contrasts have been observed between the plants of the uplands and the lowlands of India, Hawaii, Ceylon, and other tropical and subtropical areas.

The correlations between leaf-margin and environment would undoubtedly be even more striking, if it were possible to study the "vegetation" of the temperate and tropical zones rather than their "flora." That is to say, if it were possible to deal with numbers of individuals rather than species. For example, the few entire-leaved woody plants in non-xerophytic cold-temperate environments and the comparatively limited number of non-entire woody species in lowland-tropical regions are represented in most cases by a relatively limited number of individuals. Those typical cold-temperate and tropical species that are most important numerically have, except in a few instances, leaves and leaflets with entire margins in lowland-tropical regions and non-entire margins in mesophytic cold-temperate ones.

A second point that deserves consideration, in a discussion of the significance of the percentages given in Table I, is the difference in the relative adaptability of trees, shrubs, and herbs. Arborescent plants, owing to their large size and persistent aerial stems, are more directly exposed to prevailing climatic influences than are small shrubs and herbs. They are, therefore, less adaptable, and, owing to a longer interval between seed germination and seed production, can migrate less rapidly. Herbs, in marked contrast to trees, are the most adaptable and variable type of vegetation. This is due, in part to their small size which enables them to take advantage of local variations in environment, in part to the brevity of their life cycle which increases their opportunity for migration and variation, and largely to the fact that they can avoid periods of unfavorable climatic conditions underground or as small resistant seeds. Thus, although the bulk of herbaceous Dicotyledons are in all probability of comparatively recent origin, they have migrated very rapidly and have established themselves in most regions of the earth.[3]

It is not at all suprising, therefore, that the correlations between leaf-margin and climate are somewhat less strikingly shown among small shrubs and herbs than they are among arborescent species. In tropical lowlands, the smaller plants can escape the full effects of intense heat and sunlight; and it is significant that non-entire species are usually small trees, shrubs, climbing plants, and herbs, many of which occur in protected, comparatively cool habitats. On the other hand, the leaves of the dominant Dicotyledons of tropical forests are almost always entire. Within the temperate zones, not only do small shrubs and herbs live in those arid and unfavorable environments where arborescent plants are of infrequent occurrence, but in mesophytic situations may avoid the full effects of climatic influences to which large trees and shrubs are directly exposed. Of course, herbaceous types may appear above ground only during the warmer or moister seasons of the year.

In view of these facts, it appears to be highly improbable that the present distribution of entire and non-entire Dicotyledonous leaves and leaflets is largely due to factors of heredity rather than those of environment. If leaf form is little subject to modification by environment and is very firmly held on by heredity, the existing ratios between the two types of leaf-margins must have been determined by the original location, subsequent migrations, etc., of those families or groups of Dicotyledons that developed entire and non-entire leaves and leaflets. But, as is shown in the next table, the majority of the families of the Dicotyledons possess both types of foliage. Furthermore, in the distribution of the woody representatives of Dicoty-

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[3] Sinnott, E. W. and Bailey, I. W. The origin and dispersal of herbaceous Angiosperms. Annals of Botany **28**: 547–600. 1914.

TABLE IV[4]

	Woody Non-entire	Woody Entire	Herbs Non-entire	Herbs Entire
Piperaceae	....	150	....	94
Salicaceae	136	20	....	....
Juglandaceae	16	5	....	....
Betulaceae	28	....	....	....
Fagaceae	49	56	....	....
Urticales	174	80	130	52
Proteales	321	391	....	....
Olacaceae	....	69	....	....
Aristolochiaceae	....	21	1	72
Polygonaceae	2	91	22	350
Chenopodiaceae	2	153	91	207
Amarantaceae	1	83	3	248
Nyctaginaceae	....	56	3	30
Phytolaccaceae	....	13	....	23
Portulacaceae	....	5	....	60
Caryophyllaceae	....	109	7	966
Ranunculaceae	48	5	530	95
Berberidaceae	30	3	12	....
Menispermaceae	....	75	2	1
Magnoliaceae	1	36	....	....
Anonaceae	....	279	....	....
Myristicaceae	....	74	....	....
Monimiaceae	11	9	....	....
Lauraceae	1	554	1	2
Papaveraceae	....	....	106	....
Cruciferae	24	120	808	558
Resedaceae	2	3	31	30
Crassulaceae	6	57	46	200
Saxifragaceae	79	10	129	55
Pittosporaceae	9	33	8	....
Cunoniaceae	17	....	....	....
Hamamelidaceae	9	10	....	....
Rosaceae	479	187	307	22
Connaraceae	....	70	....	4
Leguminosae	13	3,329	243	2,339
Geraniaceae	63	4	294	33
Oxalidaceae	....	8	8	388
Linaceae	....	25	2	59
Zygophyllaceae	4	30	3	50
Rutaceae	77	434	20	41
Simarubaceae	6	45	....	....
Burseraceae	2	38	....	....
Meliaceae	21	176	....	....
Malpighiaceae	3	256	....	....
Polygalaceae	....	126	....	160
Euphorbiaceae	445	514	128	188
Coriariaceae	....	37	....	....
Celastraceae	107	51	....	....
Anacardiaceae	49	154	....	....

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[4] Based upon analyses of the floras of the following regions: E. C. North America, West Indies, Brazil, Patagonia, England, Spain, Flora Orientalis, Flora Capensis, western Australia, East Indies, Simla.

TABLE IV—*Continued.*

	Woody Non-entire	Woody Entire	Herbs Non-entire	Herbs Entire
Aquifoliaceae	28	30		
Aceraceae	17	1		
Sapindaceae	236	172	10	
Rhamnaceae	70	151		
Vitaceae	109	7	4	
Tiliaceae	114	38	33	
Malvaceae	162	58	300	12
Sterculiaceae	202	132	20	1
Dilleniaceae	56	58	8	
Ochnaceae	53	20		
Marcgraviaceae		15		
Guttiferae		94		
Dipterocarpaceae	3	69		
Cistaceae		96		31
Bixaceae	93	21		
Violaceae	47	16	115	22
Flacourtiaceae	10	11		
Passifloraceae	22	18	33	33
Begoniaceae	25	1	63	1
Thymeleaceae		111		3
Eleagnaceae		16		
Lythraceae	1	84		101
Rhizophoraceae		21		
Combretaceae		111		
Myrtaceae	34	1,613		
Melastomataceae	49	253	7	9
Oenotheraceae	6	16	94	78
Araliaceae	39	37		5
Umbelliferae	41	9	1,195	219
Cornaceae	4	41		
Ericaceae	89	813		
Myrsinaceae	35	107		
Primulaceae		1	69	91
Plumbaginaceae	1	105	5	101
Sapotaceae		115		
Ebenaceae		78		
Symplocaceae	24	1		
Styracaceae	6	31		
Oleaceae	17	69		
Apocynaceae		272		15
Asclepiadaceae		623		207
Convolvulaceae	43	193	102	268
Polemoniaceae			15	28
Borraginaceae	21	153	27	531
Verbenaceae	182	118	53	26
Labiatae	310	138	1,067	224
Solanaceae	29	150	100	67
Scrophulariaceae	113	113	884	396
Bignoniaceae	16	294	3	
Gesneriaceae	49	53	60	17
Acanthaceae	47	182	40	232
Myoporaceae	10	24		
Plantaginaceae		4	49	71

TABLE IV—*Continued.*

	Woody Non-entire	Woody Entire	Herbs Non-entire	Herbs Entire
Rubiaceae	4	1,261	2	610
Caprifoliaceae	39	59	8	7
Valerianaceae	1	1	70	26
Dipsacaceae	1	....	119	33
Cucurbitaceae	4	....	253	33
Goodeniaceae	14	15	38	28
Compositae	845	1,196	3,164	1,471

ledonous families, there is in almost all cases an obvious correlation between leaf form and environment. For example, such typical entire leaved woody groups as the Anonaceae, Lauraceae, Ebenaceae, Guttiferae, Rhizophoraceae, Myristicaceae, Sapotaceae, Apocynaceae, etc., are practically absent from mesophytic cold-temperate regions, as are such characteristically non-entire families as the Betulaceae, Aceraceae, Platanaceae, etc., from lowland-tropical areas. Particularly significant, however, is the distribution of those families, Malvaceae, Rosaceae, Ulmaceae, Fagaceae, Tiliaceae, Leguminosae, etc., which possess both types of leaf-margins. The non-entire types usually reach their optimum development in mesophytic temperate, cool upland, or equable environments, the entire types in lowland-tropical or physiologically dry habitats, and the transitional forms in intermediate environments. To endeavor to explain all these correlations between leaf form and environment as mere coincidences would be very difficult. When it is taken into consideration, accordingly, that correlations between leaf form and environment occur in numerous families, genera, and even species, and in all parts of the tropical, temperate, and frigid zones, the effects of environment are clearly demonstrated.

Although the form of the leaf-margin of Dicotyledons appears to be very strongly influenced by environment, historical factors and those influences of heredity which tend to maintain existing characters, are, of course, by no means inoperative. In any region, not all species will have been subject to the effects of prevailing climatic conditions for equal lengths of time or an equal number of generations; nor is it necessary to suppose that all species or groups of plants will respond with equal rapidity or in an exactly similar manner to influences of environment. Thus, the limited number of non-entire leaved types in lowland-tropical environments and the comparatively few entire-leaved species in mesophytic cold-temperate regions may be types which (1) have avoided the customary effects of prevailing climatic and edaphic influences by hidden ecological or physiological means, or (2) have been subjected to the modifying influences of a new habitat for too limited a period of time for factors of environment to neutralize those of heredity (as, for example, natural selection tending, more or less rapidly, to eliminate unfavorable forms; variations produced by heterogenesis, orthogenesis, or the inheritance of acquired characters).

Numerous illustrations of the effects of historical factors, such as migration, isolation, etc., have been encountered in tracing the distribution of the two types of leaf-margins. For example, the low percentages of entire-leaved arborescent plants in cold-temperate Eurasia, as compared with similar regions in North America, are due in all probability to the extermination of many of these forms during the glacial period; and to the fact that mountain ranges and other barriers have prevented southern types from migrating northward.

In this connection, it is interesting to note the following comparison between the native plants and the naturalized exotics that are recorded in Britton and Brown's flora of the northern United States and Canada. Here are included species from as far south as southern Virginia, Kentucky, and Kansas. The close similarity between the percentages in the two floras seems to indicate that the effects of the glacial period have been largely neutralized in North America.

TABLE V

Britton and Brown's Flora (2,365 Native Species)			Britton and Brown's Flora (501 Naturalized Exotics)		
	Percent	Entire, Percent		Percent	Percent
Trees.......	6	24	Trees.........	3	16
Shrubs	14	35	Shrubs........	7	44
Herbs......	80	49	Herbs........	90	42

As might naturally be expected, historical influences play a more important role in intermediate types of environments. For example, the high percentages of entire leaved woody plants in Tasmania and southern Australia, Table I, may be due in part to environment, but it should be remembered that these regions have been long isolated, and are not in close contact with extensive mesophytic cold-temperate regions as are the warm-temperate areas of the northern hemisphere.

Among herbaceous plants, historical factors appear to have had a more important effect upon the distribution of the two principal types of leaf-margins than they have among arborescent and shrubby Dicotyledons. This seems to have been due to the fact that, owing to the fundamental differences between these growth forms, herbaceous plants are less subject to or react differently toward prevailing environmental influences; and to the more recent origin and rapid dispersal of herbs.

What then is the physiological significance of the entire and of the non-entire types of Dicotyledonous leaf-margins? Are they actually of vital functional importance or merely necessary concomitants of certain types of foliar structure? This is clearly a problem for anatomical and experimental investigation, and will be considered separately in a subsequent paper. However, there are one or two suggestive facts that should be noted at this time. The serrations, dentations, etc., of non-entire leaves, which are often more conspicuous in the earlier than in the later stages of the ontogeny of the leaf, are frequently glandular or provided with hydathodes or water stomata. The structure and possible excretory function of the non-entire leaf-margin, therefore, deserve careful investigation.

Among woody plants, well-developed non-entire margins occur commonly on comparatively thin, soft leaves with prominent veins. Entire margins, on the other hand, usually occur on thicker, stiffer, more leathery leaves which are provided with structures that seem to retard evaporation and transpiration. The possibility suggests itself, accordingly, that the form of the leaf-margin may be largely influenced, either directly or indirectly, by phenomena of evaporation and transpiration. In those environments where non-entire margins reach their optimum development, the leaves can draw upon abundant soil moisture and transpire freely.[5] In alpine and arctic regions, bogs, steppes, prairies, moors, arid, and saline habitats the leaves of most plants with persistent aerial stems and of many herbaceous forms are exposed to conditions of physiological drought, and are subject to the grave danger of excessive transpiration and evaporation.

If this is the case, why are there such high percentages of entire-leaved plants in tropical rainforests and in other moist tropical environments? Although the leaves of woody plants in such situations are comparatively large, they appear to be somewhat xerophilous in structure. The possible necessity for the reduction of transpiration in even the most humid of lowland-tropical rainforests is indicated by the extreme effects upon foliage of even a few hours' exposure to the full intensity of tropical sunlight. Schimper states:[6] "Every visitor to the botanic gardens at Buitenzorg knows that many plants, during the later hours of the generally sunny forenoon, usually exhibit clear signs of incipient wilting; this continues to increase rapidly until the occurrence of the afternoon shower of rain, by which time many leaves hang down quite in a drooping condition, although they are not unprovided with contrivances against transpiration. During my visit to Buitenzorg in the midst of the rainy season, fourteen rainless sultry days passed in rapid succession, and the vegetation presented a parched appearance such as would hardly have arisen in Europe after a period three times that length. The air remained very moist throughout this dry period, and, in a less sunny climate, the rich nightly dew would not have been so ineffective."

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[5] It is interesting to note that in regions with marked alternating periods of heat and cold or dry and wet seasons, the evergreen foliage may be entire when the deciduous types are strikingly non-entire.

Although the usual effect of the danger of excessive transpiration seems to be to produce xerophilous leaves with entire margins, there are a number of apparent exceptions to this rule. In the sclerophyllous woodlands of warm-temperate regions, the marked tendency for the reduction in leaf surface may result (*Proteaceae, Cunoniaceae, Quercus ilex* L., *Prunus ilicifolia* Walp., etc.) in the development of highly specialized spinosely toothed, pinnatifid, or deeply divided, leathery leaves. Furthermore, certain of the softer leaved xerophytes, that are protected against excessive transpiration by hairy coverings of various sorts, have non-entire margins.

Pinnatifid or deeply divided leaves, which are of comparatively infrequent occurrence among woody plants, are more common among herbs, and occur to some extent in more or less physiologically dry habitats. In such situations, however, they usually differ from their close relatives in mesophytic habitats in having fewer or no irregularities, serrations, dentations, etc., on the margins of their lobes and sinuses.

Having studied the distribution of entire and of non-entire leaves and leaflets in existing Dicotyledonous floras, it is desirable to examine a few Cretaceous and Tertiary floras. As is well known, herbaceous Dicotyledons are of very infrequent occurrence as fossils below the upper Tertiary. In fact, the bulk of the leaves, of which we have a fossil record, seem to have belonged to arborescent or comparatively large woody species. Therefore, a study of the leaf-margins of Cretaceous and of Tertiary floras of Dicotyledons should afford a rough index of the general climatic conditions which prevailed in the region where the floras existed.

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[6] Schimper, A. F. W. Plant Geography, p. 220, English Edition, Clarendon Press, Oxford, 1903.

TABLE VI

Tertiary Floras

	Entire, Percent
Florissant, Kirchner, Upper Miocene	33
Green River, Lesq., Upper Eocene	29
John Day Basin, Knowlton, Upper Eocene	28
Spitzbergen, Heer, Upper Eocene	46
Arctic, Heer, Upper Eocene	29
Bad Lands, Lesq., Lower Eocene	29
Wilcox, Berry, Lower Eocene	83

Cretaceous Floras

	Entire, Percent
Montana, Knowlton	62
Patoot, Arctic, Heer	51
Atane, Arctic, Heer	81
Dakota, Lesq.	54
Raritan, Berry	71

A comparison of the first six Tertiary percentages, given in this table, with those of modern floras indicates very clearly the general temperate character of the climates that prevailed in the regions where these fossil floras existed. Similarly, the percentages of non-entire leaves in the Patoot and Dakota Cretaceous formations denote conditions intermediate between those of typical lowland-tropical and mesophytic cold-temperate climates. The Wilcox flora which, as has been shown by Berry, was a tropical strand flora, is in marked contrast to the more northern or temperate Tertiary floras. The high percentages of entire-leaved forms (megaphyllous) in the Atane beds points to the tropical character of the climate that existed in certain arctic regions during parts of the Cretaceous.

Of course, caution is needed in comparing any percentage in this table with that of a living flora. This is due to the fact that one cannot always be certain that any known fossil flora is a fair sample of the total ancient vegetation of which it once formed a part. Furthermore, the entire-leaved portion of a flora may contain a greater or less number of large- and small-leaved types, depending upon the exact nature of the climatic and edaphic influences that may have been operative. Therefore, in comparing fossil with living floras, it is important to take into consideration the number of megaphyllous and of microphyllous types that are represented. It should be noted, however, that this method of studying the climates of the Cretaceous and Tertiary rests upon a physiological and ecological basis rather than upon the usual phylogenetic one. It promises to afford a simple and rapid means of gauging the general climatic conditions of the Cretaceous and Tertiary, and of checking the accuracy of conclusions derived from other lines of evidence.

In conclusion, it may well be asked, what bearings this study of the distribution of entire and non-entire leaves and leaflets has upon the question of the relative conservatism of the leaf? As far as the leaf-margin of Dicotyledons is concerned, it may be very variable or extremely inconstant. For example, so long as entire-leaved plants remain in habitats that strongly favor the formation of entire margins, foliar form will remain unaltered, and the leaf will appear to be quite conservative. The most variable conditions, on the other hand, seem to occur in intermediate environments, or among entire leaved plants in non-entire environments, or *vice versa*.

It cannot be too strongly emphasized that there is grave danger in inferring that, because a certain character has remained unaltered in one or more groups of plants through long periods of geological time or has varied greatly among certain closely related forms, the organ which possesses it is inherently "conservative" or "inconstant." In any organ, not all characters are necessarily equally stable or variable. Furthermore, the same character may vary in its constancy or instability in different environments, in different plants, and at different stages in the ontogeny of the individual or in the phylogeny of the genus or family. Although characters of little or no apparent functional importance have been shown in certain cases to be more conservative than others, that are subject to the influences of environment, it should be kept in mind that a functionally important character may long remain unaltered, if it is subjected to an unchanging environment. Continuity of similar environmental influences is responsible, in all probability, for some of the most striking cases of the persistence of Dicotyledonous foliar types from the Cretaceous to the present.

The problem of the relative conservatism of the various organs or parts of plants is such an exceedingly complicated one, that it deserves careful experimental investigation.

SUMMARY

There is a very clearly marked correlation between leaf-margin and environment in the distribution of Dicotyledons in the various regions of the earth.

Leaves and leaflets with entire margins are overwhelmingly predominant in lowland-tropical regions; those with non-entire margins in mesophytic cold-temperate areas.

In the tropical zones, non-entire margins are favored by moist uplands, equable environments, and protected, comparatively cool habitats; in the cold-temperate zones, entire margins are favored by arid environments and other physiologically dry habitats.

Correlations between leaf-margin and prevailing climatic influences are more strikingly shown among trees and large shrubs than among herbs, as might naturally be expected, when the fundamental differences between these important growth forms are taken into consideration.

The determination of the percentages of entire and of non-entire leaves in Cretaceous and Tertiary Dicotyledonous floras, affords a simple and rapid means of gauging the general climatic conditions which existed in the regions where these plants flourished.

There is grave danger in inferring, because a certain foliar character has remained unaltered through long periods of geological time, or has varied greatly among closely related forms, that the leaf is inherently "conservative" or "inconstant."

The writers wish to express their sincere thanks to their colleagues in the Arnold Arboretum and Gray Herbarium for many courtesies during this investigation. To Dr. F. H. Knowlton of the United States Geological Survey and Dr. E. W. Berry, they are much indebted for valuable suggestions in regard to fossil floras.

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A GYMNOSPORANGIUM WITH REPEATING SPORES[1]

J. C. Arthur

The group of rusts now known under the generic designation of Gymnosporangium was the first to receive attention from systematic botanists. The earliest taxonomic record of any rust is that of a species on Juniperus described and figured by the pre-Linnaean author Micheli, to which he gave the generic name Puccinia. For a time this name was used for all rusts having the same general form of teliospore, then became shunted over to rusts of the general characteristics of *Puccinia graminis*, while the name Gymnosporangium was applied to the cedar rusts.

It has been easy to recognize the members of this latter genus in the telial stage by the gelatinous matrix arising from the pedicels of the teliospores coupled with occurrence on Juniperaceae, and in the aecial stage by a distinctive roestelioid peridium enclosing spores with colored walls and evident germ-pores, coupled with occurrence on Malaceae. These generic characters have been taken to indicate a highly specialized development parallel to the group represented by *Puccinia graminis*.

The genus Gymnosporangium has also been especially notable among the rusts by the utter absence of a repeating stage, either of uredinoid or aecidioid nature.

The bridging of the gap between the two groups of rusts was recently begun by finding aecia lacking the roestelioid characteristics and possessing all the features of a true Aecidium such as belong with rusts of the *P. graminis* group, as illustrated by *Aecidium Blasdaleanum* of the Pacific coast, found by cultures to go to *Gymnosporangium Libocedri*.[2] This aecidioid form, however, showed its Gymnosporangial relationship by occurring on the Malaceous genera Amelanchier and Crataegus.

A further advance was made in finding gradations between the two groups when Gymnosporangial aecia were found on the Rosaceous host Porteranthus,[3] a herbaceous plant somewhat of the habit of Agrimonia. This species was *G. exterum.* The gap was next narrowed by cultures of *G. speciosum* on Philadelphus,[4] belonging to the Hydrangiaceae, and the latest advance was made by using telia of *G. Ellisii* and growing aecia on the far more removed host genus Myrica,[5] belonging to the family Myricaceae.

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[1] Presented before the American Phytopathological Society at the Philadelphia meeting, January 1, 1915.

[2] Arthur, Cultures of Uredineae in 1908. Mycologia 1: 252. 1909.

So far as the aecial stage is concerned a complete alliance between the two groups of rusts has been established, shown in both morphological structure of the fungus and relationship of hosts. So far as the telial stage is concerned no species has yet been found in which the initial diagnostic characters of gelatinized pedicels and Juniperaceous hosts do not occur; that is to say, no advance has been made on the telial side in joining the two groups of rusts.

We now turn to a consideration of the curious lack of a repeating stage. It might be inferred that the occurrence of the sporophyte on gymnospermous hosts indicates an ancient segregation of the group, which during a long course of specialization has dropped out the repeating stage. Furthermore, there may be something inhibitive in the nature of the gymnospermous host, although it would be difficult to guess what it might be. Or, the long-lived sporophytic mycelium, often persisting for many years and never quite annual, may have rendered the repeating stage unnecessary, and thus led to its suppression.

It has been suggested that the repeating stage occurs on the aecial host, being aecidioid, similar to that in *Puccinia ambigua* on Galium. The failure to find what might be considered secondary Aecidia, that is Aecidia unaccompanied by pycnia, seemed to negative this view. Nevertheless, more than one attempt has been made to infect the aecial host by sowing aeciospores, but uniformly without success.

For some years an apparently genuine Uredo of the general form of that belonging to *P. graminis* has been known on a Juniperaceous host. In 1899 while on the Harriman Expedition to Alaska Professor Trelease obtained a small amount of what he named *Uredo nootkatensis*[6] on *Chamaecyparis nootkatensis*, the yellow or Alaska cedar of the

---

[3] Arthur, Cultures of Uredineae in 1908. Mycologia **1**: 253. 1909.

[4] Arthur, Cultures of Uredineae in 1911. Mycologia **4**: 63. 1912.

[5] Fromme, A new Gymnosporangial connection. Mycologia **6**: 226. 1914.

[6] Trelease, in Alaska. Harr. Exped. **5**: 36. 1904.

Pacific Coast. It was found at the hot springs on Baranof Island in southeastern Alaska. When I began studying the material of this collection, through the kindness of Professor Trelease, I felt confident that a knowledge of the complete life history would throw light upon the evolution or affinities of the Juniperaceous rusts. As the collection was made the middle of June it seemed probable that telia might be found on the same host later in the season. I wrote to Professor C. C. Georgeson of the Experiment Station at Sitka, Baranof Island, and to other Experiment Station men in Alaska, and to botanists going to Alaska, asking them to look out for such a hypothetical rust, but without returns. When Dr. Kern prepared his monograph on the genus Gymnosporangium in 1911, he made good use of the ideas brought together up to that time, and predicted that telia would eventually be found of the foliicolous form, causing no hypertrophy. He also predicted that *Aecidium Sorbi* would be shown to be the aecial stage.[7]

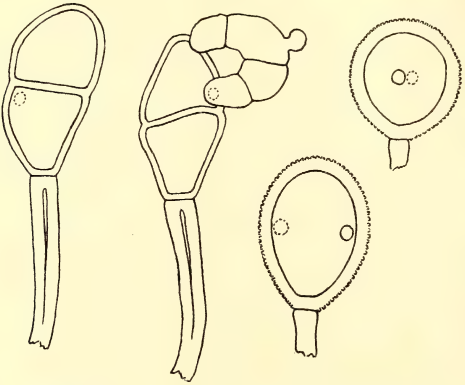

FIG. 1. Two teliospores, one germinating, and two urediniospores of *Gymnosporangium nootkatensis*. Drawn with camera lucida from material sent by Prof. H. S. Jackson from Oregon. × 625.

The collection, which finally gave the key to the problem, came from Professor H. S. Jackson of the Oregon Agricultural College, having been made by H. P. Barss and G. B. Posey August 15, 1914, on the north slope of Mount Jefferson, along the trail to Hanging Valley, at an altitude of 5,000 feet. The green sprays of *Chamaecyparis nootkatensis* were abundantly dotted beneath with the conspicuous, golden-yellow sori of the rust. Upon microscopic examination the sori were found to contain many teliospores, some of which were in process of germination at time of gathering (fig. 1), although six weeks later, when they had reached me, both urediniospores and teliospores had lost their viability. The teliospores in a sorus probably did not exceed one teliospore to a hundred urediniospores, the condition apparently being that of the early stage in the transformation of a uredinial into a telial sorus.

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[7] Kern, A biologic and taxonomic study of the genus Gymnosporangium. Bull. N. Y. Bot. Gard. 7: 408. 1911.

It still seems probable that true telial sori will eventually be found. There would, however, evidently be no difficulty in producing the aecial stage from such teliospores as those found in the present instance; and cultures could confidently be attempted with similar material, should circumstances favor.

It is now some five years since Dr. Kern[8] suggested "the possibility of a relationship between the cedar-rust, *Uredo nootkatensis*, and *Aecidium Sorbi*." This prediction was supported by "inferences drawn from analogy, homology and geographic distribution," as he explained in his monograph (p. 408) of the following year. Without taking time to review the grounds of the argument, it may be said that time has added items of strength, without detecting flaws. The argument from geographic distribution has the present basis. The following list embraces all collections now known to the writer:

Alaska, Baranof Island: I on *Malus rivularis*, Aug. 2, 1914, *J. P. Anderson*, on *Sorbus scopulina*, Aug. 30, 1897, *C. S. Sargent*, on *S. sitchensis*, Aug. 2, 1914, *J. P. Anderson*, II on *Chamaecyparis nootkatensis*, June 15, 1899, *Wm. Trelease.*

British Columbia, Vancouver Island: I on *Sorbus occidentalis*, Aug. 8, 1905, *F. K. Butters*, on *M. rivularis*, Aug. 4, 1907, *F. K. Butters.*

Washington, Mount Rainier (Tacoma): I on *S. occidentalis*, Aug. 24, 1901, *E. W. D. Holway*, II on *C. nootkatensis*, August, 1913, *C. von Tubeuf*;—Goat Mountains (near Mt. Rainier): I on *S. occidentalis*, Sept. 11, 1895, *J. A. Allen*;—Olympic Mountains: I on *S. occidentalis*, June, 1900, *A. D. E. Elmer*, Aug. 15, 1907, *T. C. Frye.*

Oregon, Mount Jefferson: II and III on *C. nootkatensis*, Aug. 15, 1914, *H. P. Barss* and *G. B. Posey.*

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[8] Kern, Prediction of relationships among some parasitic fungi. Science **31**: 833. May, 1910.

It will be seen from this list that there is a remarkable coincidence in the distribution of the two forms. The range of the cedar-rust is nearly co-extensive with the range of the host, but the similar restriction of the Malaceous rust can not depend upon the aecial hosts, which are far more widely distributed. To make the geographic argument even more convincing it should be stated that no other unattached Gymnosporangial aecia or telia are known from the region in question.

As the teliospores now found to be associated with the uredinia upon *Chamaecyparis nootkatensis* are of the general form and structure of certain other species of Gymnosporangium to be considered closely related, and as the structure and appearance of the uredinia conform to theoretical demands based upon analogy with the large group of rusts represented by *Puccinia graminis*, and furthermore as both structure and geographic distribution assure the probable association of *Aecidium Sorbi* as the alternate stage, it seems fitting to present the following synonymy and description of the species in its entirety.

Gymnosporangium nootkatensis (Trelease) comb. nov.

Uredo nootkatensis Trelease, Alaska Harr. Exped. **5**: 36. 1904.
Aecidium Sorbi Arthur, Bull. Torrey Club, **33**: 521. 1906.
Gymnosporangium Sorbi Kern, Bull. N. Y. Bot. Gard. **7**: 438. 1911.
Uredo Chamaecyparidis-nutkaensis Tubeuf, Nat. Zeits. Forst.-Landw. **2**: 91. 1914.

O and I. Described in N. Amer. Flora **7**: 190–191. 1912.

ON MALACEAE:

Malus rivularis (Dougl.) Roem. Alaska, British Columbia.
Sorbus occidentalis (S. Wats.) Greene, Washington; British Columbia.
Sorbus scopulina Greene, Alaska.
Sorbus sitchensis Roem. Alaska.

II. Uredinia foliicolous, on yellow spots covering the whole leaf, subepidermal, round, 0.5 mm. across, bright orange fading to light yellow, prominently pulvinate, somewhat pulverulent, ruptured epidermis generally noticeable; urediniospores globose or obovate-globoid, 28–32 μ in diameter; wall pale yellow becoming colorless, moderately thick, 3–4 μ, finely and closely verrucose, appearing radiately striate, the pores 2, equatorial; pedicels colorless, somewhat persistent.

III. Telia not seen; teliospores in the uredinial sori 2-celled, ellipsoid, 23–29 by 42–48 μ, narrowed below, rounded or narrowed above, slightly or not constricted at the septum; wall pale lemon-yellow, uniformly thin, 1–1.5 μ, smooth, the pores one in each cell near the septum; pedicel colorless, evenly thick, 5–7 μ, once to twice length of spore.

ON JUNIPERACEAE:

Chamaecyparis nootkatensis (Lamb.) Spach, Oregon, Alaska.

Distribution: From southeastern Alaska to northwestern Oregon, in cool localities upon the sea coast northward or 5,000–6,000 feet elevation southward.

A few words only need be said to point out that the completion of the life-cycle of this one species makes a long stride forward in firmly establishing the theoretical basis upon which the classification of the Uredinales presented at Vienna in 1905 was constructed.[9] That classification undertook to consider all rusts from the standpoint primarily of their life-histories, actual or potential, and secondarily of their comparative morphology. From lack of knowledge much had to be assumed, but as the passing years supply the desired information, the reasonableness of the original assumptions becomes more and more apparent.

According to this classification all rusts are either short-cycled, having only one sporophytic spore-form, or long-cycled, having (1) a primary (aecial) spore-form, (2) a repeating stage, in some species partly or wholly suppressed, and (3) a final (telial) spore-form. The cedar-rusts presented a highly differentiated group, possibly the most specialized of all the rusts, in which no repeating-spores were known. So long as the repeating-stage was unknown, some doubt attached to the true position of the group in the classification, and even more doubt to the genuineness of the basic assumptions of the whole scheme. Bringing to light this primitive species of Gymnosporangium with its very active repeating stage is, therefore, a matter of moment.

PURDUE UNIVERSITY,
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[9] Arthur, Eine auf die Struktur und Entwicklungsgeschichte begründete Klassification der Uredineen. Résult. Sci. Congr. Bot. Vienne 331. 1906.

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THE EXCHANGE OF IONS BETWEEN THE ROOTS OF *LUPINUS ALBUS* AND CULTURE SOLUTIONS CONTAINING THREE NUTRIENT SALTS[1]

RODNEY H. TRUE AND HARLEY HARRIS BARTLETT

Elsewhere in this Journal the authors have given data on root absorption from solutions containing a single nutrient salt,[2] and also from mixed solutions of two salts.[3] It may be recalled that the roots of young seedlings of *Lupinus albus* actively absorb calcium salts. Magnesium salts are absorbed to a less extent and only from solutions which are so weak that toxic action does not occur. Potassium and sodium stand in marked contrast to calcium and magnesium. Their solutions greatly resemble distilled water in that the roots placed in them either excrete electrolytes regardless of the concentration or else effect a minimal absorption. The somewhat different effect of different salts of the same base indicates that absorption is influenced not only by the cation but also by the anion.

The addition of even a very small amount of a calcium salt to a solution of a magnesium salt increases absorption to a remarkable degree. The same effect is also observed when calcium is added to a potassium salt. In mixtures of two nutrient nitrates there usually seems to be a ratio which is more favorable to absorption than any other. In certain cases where the total salt concentration was 240 $N \times 10^{-6}$ the absorption from mixtures was found to be greater than from either constituent salt alone. In weaker solutions

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[1] Published by permission of the Secretary of Agriculture.

[2] True, R. H., and Bartlett, H. H., The Exchange of Ions between the Roots of *Lupinus albus* and Culture Solutions Containing One Nutrient Salt. Amer. Journ. Bot. **2**: 255–278. 1915.

[3] True, R. H., and Bartlett, H. H., The Exchange of Ions between the Roots of *Lupinus albus* and Culture Solutions Containing Two Nutrient Salts. Amer. Journ. Bot. **2**: 311–31. 1915.

[The **Journal** for January (**3**: 1–46) was issued Feb. 5, 1916.]

the absorption was more likely to be a mean between that of the unmixed constituent salts at the same total concentration.

The present paper deals primarily with absorption from mixtures of three salts. Two experiments are reported, one of which was carried out with the nitrates of potassium, calcium and magnesium, the other with monopotassium phosphate, calcium nitrate and magnesium sulphate. In each experiment 36 culture solutions were used, the uniform concentration of which was 140 $N \times 10^{-6}$. Monopotassium phosphate was treated as though it were a salt of a univalent acid, on account of the fact that it dissociates for the most part in K^+ and $H_2PO_4^-$ ions. The 36 solutions provided all the possible combina-

TABLE I.

Composition			Daily Concentration				
KNO_3	$Ca(NO_3)_2$	$Mg(NO_3)_2$		1	2	3	4
140 $N \times 10^{-6}$	0 $N \times 10^{-6}$	0 $N \times 10^{-6}$	1.000	1.033	1.043	1.077	1.086
120 “	20 “	0 “	1.000	1.027	1.036	1.037	1.036
120 “	0 “	20 “	1.000	1.027	1.043	1.048	1.040
100 “	40 “	0 “	1.000	1.032	1.045	1.018	1.021
100 “	20 “	20 “	1.000	1.063	1.011	0.934	0.894
100 “	0 “	40 “	1.000	1.048	1.054	1.039	1.022
80 “	60 “	0 “	1.000	1.061	1.087	1.059	1.038
80 “	40 “	20 “	1.000	1.065	1.073	1.039	1.019
80 “	20 “	40 “	1.000	1.041	1.052	1.042	1.036
80 “	0 “	60 “	1.000	1.041	1.057	1.046	1.030
60 “	80 “	0 “	1.000	1.038	1.053	1.034	1.029
60 “	60 “	20 “	1.000	1.065	1.035	0.943	0.878
60 “	40 “	40 “	1.000	1.061	1.001	0.901	......
60 “	20 “	60 “	1.000	1.061	1.073	1.023	1.003
60 “	0 “	80 “	1.000	1.045	1.058	1.015	0.995
40 “	100 “	0 “	1.000	1.059	1.124	1.112	1.141
40 “	80 “	20 “	1.000	1.029	1.058	......	1.046
40 “	60 “	40 “	1.000	1.033	1.027	0.948	0.915
40 “	40 “	60 “	1.000	1.055	1.083	1.058	1.052
40 “	20 “	80 “	1.000	1.048	1.043	0.988	0.964
40 “	0 “	100 “	1.000	1.042	1.058	1.036	1.015
20 “	120 “	0 “	1.000	1.062	1.098	1.084	1.093
20 “	100 “	20 “	1.000	1.054	1.058	1.003	0.983
20 “	80 “	40 “	1.000	1.069	1.088	1.043	1.037
20 “	60 “	60 “	1.000	1.083	1.141	1.147	1.169
20 “	40 “	80 “	1.000	1.014	1.003	0.921	0.889
20 “	20 “	100 “	1.000	1.022	1.030	0.963	0.939
20 “	0 “	120 “	1.000	1.018	1.018	0.982	0.969
0 “	140 “	0 “	1.000	1.027	0.980	0.919	0.875
0 “	120 “	20 “	1.000	1.019	1.007	0.949	0.916
0 “	100 “	40 “	1.000	1.007	0.996	0.930	0.890
0 “	80 “	60 “	1.000	1.040	0.996	0.920	0.880
0 “	60 “	80 “	1.000	1.031	1.001	0.948	0.910
0 “	40 “	100 “	1.000	1.054	1.039	0.982	0.954
0 “	20 “	120 “	1.000	1.030	1.039	1.000	0.986
0 “	0 “	140 “	1.000	1.017	1.036	1.025	0.998

tions of one, two, or three salts which could be obtained in which each constituent salt had a partial concentration of 20 N × 10^{-6} or some multiple of that concentration. If the composition of the 36 solutions were represented by a triangular diagram, in the manner which has become familiar to plant physiologists through the work of Schreiner and Skinner[4] the apices of the triangle would indicate the three unmixed constituent salts, the remaining peripheral positions would indicate the various mixtures of the three different pairs of

TABLE I.

Daily Concentration											
5	6	7	8	9	10	11	12	13	14	15	16
1.108	1.073	1.066	1.031	1.035	1.026	0.984	0.982	0.963	0.966	0.981	1.027
1.004	0.989	0.974	0.945	0.914	0.889	0.864	0.869	0.859	0.879	0.931	1.015
1.022	1.001	0.998	0.968	0.960	0.966	0.958	0.968	0.978	......	1.053	1.164
0.994	0.971	0.947	0.893	0.824	0.768	0.732	0.714	0.694	0.683	0.701	0.735
0.861	0.833	0.791	0.753	0.706	0.681	0.658	0.662	0.665	0.678	0.716	0.767
1.000	0.984	0.968	0.936	0.924	0.908	0.893	0.915	0.931	0.960	1.026	1.126
1.022	1.005	0.950	0.896	0.815	0.744	0.703	0.681	0.672	0.672	0.710	0.767
0.995	0.972	0.916	0.866	0.797	0.748	0.730	0.747	0.755	0.788	0.840	0.907
1.028	1.013	0.979	0.937	0.890	0.835	0.796	0.791	0.792	0.826	0.896	1.007
1.008	0.985	0.959	0.922	0.897	0.891	0.875	0.876	0.893	0.914	0.965	1.041
1.028	1.032	1.009	0.975	0.890	0.818	0.757	0.715	0.680	0.668	0.703	0.780
0.815	0.796	0.744	0.707	0.638	0.567	0.511	0.478	0.450	0.436	0.458	0.521
0.798	0.767	0.708	0.661	0.593	0.542	0.506	0.492	0.490	0.511	0.567	0.641
0.990	0.969	0.928	0.885	0.817	0.763	0.714	0.672	0.654	0.649	0.679	0.735
0.971	0.953	0.915	0.871	0.832	0.798	0.771	0.769	0.778	0.803	0.863	0.952
1.158	1.139	1.112	1.047	0.937	0.836	0.774	0.712	0.661	0.629	0.631	0.663
1.056	1.044	0.970	0.905	0.796	0.705	0.653	0.614	0.591	0.569	0.598	0.661
0.873	0.859	0.787	0.739	0.656	0.589	0.542	0.506	0.467	0.448	0.438	0.472
1.050	1.041	1.007	0.954	0.853	0.755	0.691	0.657	0.636	0.635	0.672	0.739
0.921	0.901	......	0.804	0.745	0.705	0.669	0.666	0.675	0.680	0.714	0.754
1.001	0.994	0.946	0.904	0.846	0.811	0.796	0.805	0.823	0.851	0.905	0.973
1.080	1.056	1.010	0.960	0.905	0.830	0.779	0.711	0.657	0.608	0.612	0.649
0.949	0.914	0.841	......	0.815	0.761	0.575	0.529	0.494	0.481	0.503	0.559
1.003	0.977	0.928	0.857	0.760	0.683	0.658	0.670	0.686	0.726	0.785	0.864
1.193	1.195	1.148	1.115	1.046	0.962	0.905	0.818	0.749	0.694	0.710	0.765
0.844	0.816	0.755	0.716	0.659	0.590	0.546	0.514	0.480	0.458	0.465	0.508
0.923	0.907	0.872	0.835	0.771	0.770	0.672	0.639	0.625	0.629	0.662	0.725
0.945	0.935	0.919	0.892	0.853	0.820	0.795	0.776	0.763	0.760	0.784	0.824
0.849	0.812	0.786	0.749	0.707	0.639	0.596	0.571	0.543	0.546	0.573	0.617
0.924	0.921	0.921	0.981	0.860	0.812	0.788	0.739	0.684	0.633	0.622	0.640
0.871	0.854	0.818	0.771	0.729	0.685	0.654	0.635	0.617	0.591	0.607	0.660
0.840	0.810	0.787	0.749	0.713	0.667	0.636	0.612	0.585	0.584	0.625	0.676
0.889	0.870	0.845	0.810	0.756	0.702	0.657	0.621	0.592	0.575	0.600	0.651
0.913	0.877	0.823	0.770	0.717	0.665	0.624	0.615	0.631	0.652	0.698	0.767
0.988	0.974	0.956	0.912	0.864	0.799	0.752	0.726	0.732	0.741	0.790	0.850
0.966	0.941	0.917	0.878	0.872	0.863	0.872	0.907	0.942	0.988	1.078	1.185

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[4] Schreiner, O., and Skinner, J. J., Ratio of Phosphate, Nitrate and Potassium on Absorption and Growth. Bot. Gaz. **50**: 1–30. 1910; Some Effects of a Harmful Soil Constituent. Bot. Gaz. **50**: 161–181. 1910.

salts, and the interior positions the mixtures of all three salts. The method used in carrying out the present work was exactly that detailed in previous papers. The electrical conductivity of each solution was read before the lupine seedlings were placed in it. During the progress of the experiments the conductivity and temperature of the solutions were read daily. Unfortunately temperature regulation was impossible, but the conductivities were of course reduced to a uniform temperature. The effect of temperature on absorption is still to be determined. From some of the experiments reported in our former papers it appears that the temperature effect within a limited range is not great. Since further work must be done in order to determine the precise effect of temperature on absorption, we have not included the daily temperature readings in this paper.

EXPERIMENT I. $KNO_3 + Ca(NO_3)_2 + Mg(NO_3)_2$

This experiment was carried out with the nitrates of potassium, calcium and magnesium, and lasted 16 days. The composition and daily concentration of each solution is expressed in Table I.[5] To facilitate interpretation the original concentration of each solution is taken as unity. In this way the magnitude of absorption or excretion as compared with the total original concentration is most readily made apparent. It was impossible, on account of the large number of culture solutions, to state the results intelligibly by means of curves. A concentration greater than unity indicates that excretion of electrolytes from the roots has taken place; absorption, on the contrary, is indicated if the concentration is less than unity.

In figure 1 we have stated on a triangular diagram the residual concentrations of the 36 culture solutions at the time of maximum absorption. The residual concentration is stated, as in the table, as a fraction of the original concentration. The greatest absorption was of course attained in different solutions on different days. Each point in the figure represents a solution, the original composition of which is indicated on the three intersecting axes reading upwardly from the intersection. The sum of the numerals on the three axes at any point is 140, indicating a total concentration of 140 N × 10^{-6}. For instance, the point marked 80 KNO_3, 20 $Ca(NO_3)_2$, 40 $Mg(NO_3)_2$

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[5] *Table I.*—Concentration changes in culture solutions containing KNO_3, $Ca(NO_3)_2$ and $Mg(NO_3)_2$, due to absorption and excretion of electrolytes by roots of *Lupinus albus*. The initial concentration ($140\ N \times 10^{-6}$) of each solution is represented by 1.000. The daily concentration is therefore stated as a ratio of residual concentration to initial concentration. To obtain the absolute concentration, in terms of $N \times 10^{-6}$, multiply by 140.

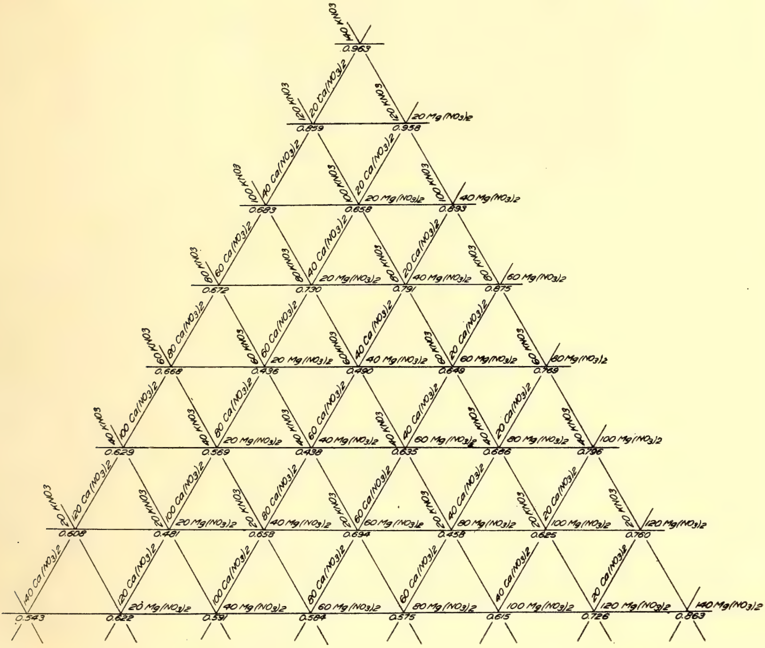

FIG. 1. Residual concentration of solutions containing KNO_3, $CA(NO_3)_2$ and $Mg(NO_3)_2$, at the time of maximum absorption.

represents a solution with a total concentration of 140 N × 10^{-6}, in which 80/140 of the NO_3^- ions were derived from KNO_3, 20/140 from $Ca(NO_3)_2$ and 40/140 from $Mg(NO_3)_2$.

Inspection of the results shows in nearly all cases a preliminary rise in the conductance of the solutions, probably due, as we have suggested elsewhere, to carbon dioxide given off by the roots. After this preliminary rise the conductance in most cases rapidly diminishes until the twelfth to fifteenth day, due to the absorption of salts by the roots. In the cases of five out of fifteen of the solutions which contained all three salts, the absorption exceeded that in the solution of $Ca(NO_3)_2$ alone. These five solutions in order of magnitude of absorption, beginning with the greatest, were as follows:

$60 N \times 10^{-6}$ KNO_3 : $40 N \times 10^{-6}$ $Ca(NO_3)_2$: $40 N \times 10^{-6}$ $Mg(NO_3)_2$,
20 " KNO_3 : 100 " $Ca(NO_3)_2$: 20 " $Mg(NO_3)_2$,
20 " KNO_3 : 40 " $Ca(NO_3)_2$: 80 " $Mg(NO_3)_2$,
40 " KNO_3 : 60 " $Ca(NO_3)_2$: 40 " $Mg(NO_3)_2$,
60 " KNO_3 : 60 " $Ca(NO_3)_2$: 20 " $Mg(NO_3)_2$.

From the remaining solutions absorption was less than from the simple $Ca(NO_3)_2$ solution. It is likely that a repetition of this experiment

TABLE II.

Composition			Daily Concentration Relations				
KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$		1	2	3	4
$140 N \times 10^{-6}$	$0 N \times 10^{-6}$	$0 N \times 10^{-6}$	1.000	1.037	1.078	1.079	1.052
120 "	20 "	0 "	1.000	0.967	0.917	0.820	0.758
120 "	0 "	20 "	1.000	1.018	1.036	....	0.973
100 "	40 "	0 "	1.000	1.003	0.969	0.879	0.812
100 "	20 "	20 "	1.000	1.005	0.952	0.849	0.783
100 "	0 "	40 "	1.000	1.012	1.005	0.958	0.902
80 "	60 "	0 "	1.000	0.994	0.942	0.864	0.797
80 "	40 "	20 "	1.000	0.976	0.942	0.835	0.760
80 "	20 "	40 "	1.000	0.974	0.903	0.779	0.691
80 "	0 "	60 "	1.000	1.005	0.983	0.910	0.890
60 "	80 "	0 "	1.000	0.997	0.959	0.873	0.821
60 "	60 "	20 "	1.000	0.985	0.931	0.825	0.761
60 "	40 "	40 "	1.000	0.977	0.899	0.764	0.683
60 "	20 "	60 "	1.000	0.972	0.887	0.755	0.674
60 "	0 "	80 "	1.000	1.021	0.997	0.923	0.876
40 "	100 "	0 "	1.000	1.022	0.965	0.903	0.834
40 "	80 "	20 "	1.000	1.022	0.947	0.830	0.761
40 "	60 "	40 "	1.000	1.008	0.945	0.826	0.747
40 "	40 "	60 "	1.000	0.993	0.933	0.810	0.727
40 "	20 "	80 "	1.000	0.997	0.932	0.798	0.706
40 "	0 "	100 "	1.000	1.037	1.034	0.986	0.957
20 "	120 "	0 "	1.000	1.034	1.009	0.946	0.908
20 "	100 "	20 "	1.000	1.005	0.930	0.808	0.752
20 "	80 "	40 "	1.000	1.016	0.934	0.806	0.734
20 "	60 "	60 "	1.000	0.997	0.920	0.775	0.705
20 "	40 "	80 "	1.000	0.999	0.901	0.749	0.652
20 "	20 "	100 "	1.000	0.997	0.913	0.730	0.624
20 "	0 "	120 "	1.000	1.023	1.007	0.951	0.929
0 "	140 "	0 "	1.000	1.075	1.083	1.051	1.040
0 "	120 "	20 "	1.000	1.061	1.050	0.999	0.970
0 "	100 "	40 "	1.000	1.053	1.011	0.929	0.868
0 "	80 "	60 "	1.000	1.035	0.996	0.886	0.827
0 "	60 "	80 "	1.000	1.039	0.988	0.871	0.807
0 "	40 "	100 "	1.000	1.014	0.971	0.885	0.831
0 "	20 "	120 "	1.000	1.012	0.959	0.865	0.816
0 "	0 "	140 "	1.000	1.029	1.019	0.950	0.927

would show somewhat different results, because the individual variation in seedlings introduces an error which is surely of considerable magnitude. The general fact is nevertheless clear that mixtures of three nitrates are more favorable to absorption than solutions of single nitrates or mixtures of two. Our former work (l. c.) on mixtures of two salts indicates that if the total concentration of the solutions had been greater, there would have been a much greater difference between the absorption from single salts and that from the less favorable mixtures. However that may be, the contrast between this experiment and the next is very great. The fact to be kept in mind is that in even the most favorable mixtures of the three nitrates

TABLE II.

Daily Concentration Relations									
5	6	7	8	9	10	11	12	13	14
1.037	0.998	0.918	0.831	0.757	0.735	0.723	0.770	0.900	......
0.659	0.576	0.533	0.499	0.466	0.464	0.511	0.607	0.857	......
0.937	0.897	0.880	0.848	0.797	0.801	0.837	0.913	1.020	......
0.712	0.619	0.531	0.444	0.370	0.360	0.401	0.510	0.761	......
0.653	0.528	0.432	0.358	0.306	0.296	0.322	0.361	0.492	......
0.824	0.723	0.665	0.629	0.591	0.614	0.708	0.869	1.186	......
0.688	0.571	0.487	0.393	0.300	0.231	0.208	0.249	0.387	......
0.578	0.517	0.432	0.345	0.263	0.198	0.176	0.198	0.301	......
0.563	0.441	0.349	0.296	0.254	0.256	0.303	0.380	0.551	......
0.805	0.728	0.672	0.640	0.607	0.595	0.618	0.661	0.753	......
0.738	0.642	0.576	0.476	0.406	0.342	0.295	0.262	0.285	......
0.660	0.551	0.464	0.400	0.349	0.310	0.290	0.298	0.363	......
0.548	0.430	0.352	0.261	0.186	0.144	0.127	0.187	0.216	......
0.549	0.441	0.356	0.298	0.243	0.211	0.195	0.198	0.267	......
0.806	0.732	0.683	0.649	0.612	0.610	0.632	0.663	0.736	......
0.748	0.675	0.640	0.576	0.502	0.445	0.402	0.379	0.403	......
0.671	0.593	0.517	0.443	0.375	0.328	0.288	0.271	0.312	......
0.589	0.441	0.344	0.276	0.206	0.160	0.143	0.137	0.190	......
0.533	0.368	0.278	0.216	0.162	0.132	0.121	0.133	0.228	0.451
0.550	0.415	0.341	0.292	0.243	0.208	0.199	0.221	0.335	0.571
0.910	0.846	0.828	0.812	0.774	0.759	0.763	0.784	0.832	0.917
0.851	0.793	0.733	0.667	0.600	0.546	0.500	0.471	0.449	0.491
0.691	0.620	0.585	0.544	0.499	0.453	0.399	0.354	0.387	0.514
0.624	0.550	0.491	0.440	0.388	0.346	0.320	0.289	0.294	0.367
0.578	0.476	0.395	0.322	0.255	0.211	0.189	0.196	0.247	0.354
0.518	0.427	0.339	0.287	0.218	0.174	0.151	0.132	0.140	0.227
0.494	0.410	0.359	0.312	0.263	0.224	0.205	0.193	0.227	0.337
0.895	0.851	0.829	0.805	0.765	0.749	0.741	0.740	0.762	0.839
1.003	0.969	0.916	0.868	0.803	0.735	0.705	0.682	0.733	0.852
0.885	0.807	0.738	0.670	0.586	0.514	0.476	0.448	0.453	0.549
0.750	0.653	0.560	0.484	0.401	0.343	0.323	0.331	0.400	0.553
....	0.653	0.599	0.528	0.452	0.406	0.379	0.361	0.400	0.534
0.699	0.614	0.534	0.464	0.395	0.332	0.305	0.291	0.339	0.456
0.734	0.650	0.590	0.541	0.489	0.468	0.472	0.489	0.547	0.657
0.733	0.665	0.616	0.576	0.525	0.493	0.474	0.464	0.513	0.660
0.920	0.831	0.822	0.807	0.781	0.794	0.805	0.835	0.888	0.978

little more than half of the salt content of the solutions had been absorbed by the roots at the time of maximum absorption. In the most favorable mixtures the concentration of Ca^{++} was electrolytically more than equivalent to the sum of $Mg^{++} + K^{+}$ and the two latter ions were either about equivalent, electrolytically, to one another, or else the K^{+} concentrations were somewhat more than equivalent electrolytically to the Mg^{++} concentration. In other words the numerical ratio of ions in the best mixtures was about 2 Ca^{++} : 1 Mg^{++} : 2K^{+}. Further experiments, however, must be carried out before any great stress is laid on this ratio as the most favorable for absorption.

EXPERIMENT 2. $KH_2PO_4 + Ca(NO_3)_2 + MgSO_4$

This experiment was carried out in order to determine the extent to which the anions influence absorption. Accordingly, cultures were grown in solutions of potassium dihydrogen phosphate (which dissociates for the most part into the univalent ions K^{+} and $H_2PO_4^{-}$), calcium nitrate and magnesium sulphate. The composition and daily concentration of each solution is stated in Table II,[6] which is in every respect comparable with Table I.

In figure 2 we have indicated the residual concentration of the 36 culture solutions at the time of maximum absorption. Inspection of the table and the figure shows important differences between the cultures containing three anions and those containing only the NO_3^{-} anion. In but three out of 15 mixtures containing all three salts did the plants fail to show absorption during the first day. This result is in marked contrast to that of experiment 1, and seems to indicate that root absorption was so active from the very start as to overbalance the effect of CO_2 excretion. Rapid absorption was maintained in most of the mixtures throughout the experiment.

The maximum absorption, attained after ten or eleven days, was in every case greater than in the corresponding nitrate solution, and was attained one or two days sooner. Whereas in the nitrate solutions only a third of the mixtures showed a total absorption greater than that from calcium nitrate alone, in the solutions with three anions all of the mixtures were superior to calcium nitrate alone. In the nitrate mixtures the most favorable solution was reduced to little below half its original concentration, but in the mixtures containing different anions the best solutions were reduced to about one

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[6] *Table II.*—Concentration changes in culture solutions containing KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$, due to absorption and excretion of salts by roots of *Lupinus albus*. The initial concentration (140 N × 10^{-6}) of each solution is represented by 1.000. The daily concentration is therefore stated as a ratio of residual concentration to initial concentration. To obtain the absolute concentration, in terms of N × 10^{-6}, multiply by 140.

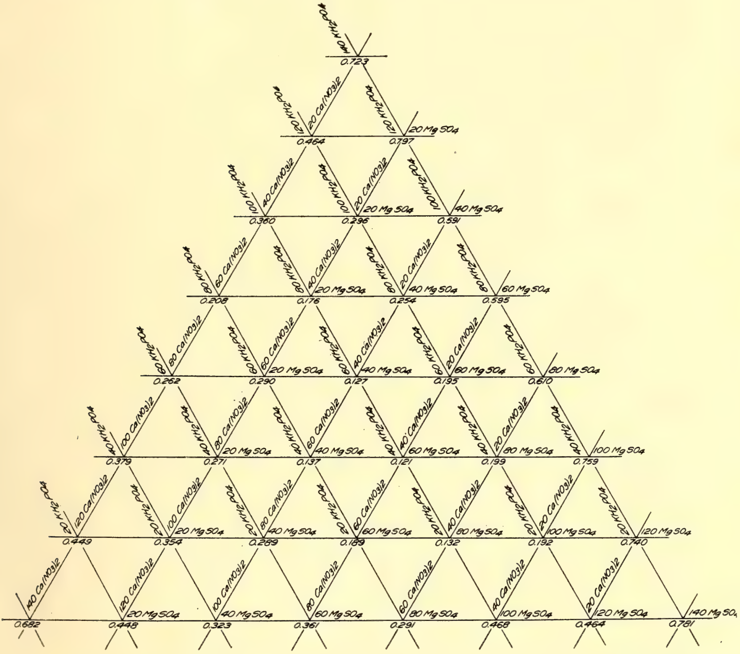
140 KH2PO4
0.723
120 KH2PO4
20 Ca(NO3)2
0.464
120 KH2PO4
20 Mg SO4
0.797
100 KH2PO4
40 Ca(NO3)2
0.360
100 KH2PO4
20 Ca(NO3)2
20 Mg SO4
0.296
100 KH2PO4
40 Mg SO4
0.591
80 KH2PO4
60 Ca(NO3)2
0.208
80 KH2PO4
40 Ca(NO3)2
20 Mg SO4
0.176
80 KH2PO4
20 Ca(NO3)2
40 Mg SO4
0.254
80 KH2PO4
60 Mg SO4
0.595
60 KH2PO4
80 Ca(NO3)2
0.262
60 KH2PO4
60 Ca(NO3)2
20 Mg SO4
0.290
60 KH2PO4
40 Ca(NO3)2
40 Mg SO4
0.127
60 KH2PO4
20 Ca(NO3)2
60 Mg SO4
0.195
60 KH2PO4
80 Mg SO4
0.610
40 KH2PO4
100 Ca(NO3)2
0.379
40 KH2PO4
80 Ca(NO3)2
20 Mg SO4
0.271
40 KH2PO4
60 Ca(NO3)2
40 Mg SO4
0.137
40 KH2PO4
40 Ca(NO3)2
60 Mg SO4
0.121
40 KH2PO4
20 Ca(NO3)2
80 Mg SO4
0.199
40 KH2PO4
100 Mg SO4
0.759
20 KH2PO4
120 Ca(NO3)2
0.449
20 KH2PO4
100 Ca(NO3)2
20 Mg SO4
0.354
20 KH2PO4
80 Ca(NO3)2
40 Mg SO4
0.289
20 KH2PO4
60 Ca(NO3)2
60 Mg SO4
0.189
20 KH2PO4
40 Ca(NO3)2
80 Mg SO4
0.132
20 KH2PO4
20 Ca(NO3)2
100 Mg SO4
0.192
20 KH2PO4
120 Mg SO4
0.740
140 Ca(NO3)2
0.682
120 Ca(NO3)2
20 Mg SO4
0.448
100 Ca(NO3)2
40 Mg SO4
0.323
80 Ca(NO3)2
60 Mg SO4
0.361
60 Ca(NO3)2
80 Mg SO4
0.291
40 Ca(NO3)2
100 Mg SO4
0.468
20 Ca(NO3)2
120 Mg SO4
0.464
140 Mg SO
0.781

FIG. 2. Residual concentration of solutions containing KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$, at the time of maximum absorption.

tenth of their original concentration. In the nitrate series there was a wide range of variation in the absorption from the 15 mixtures of all three salts, but in the mixed anion series there was much greater uniformity in the final result. The least favorable mixtures did not depart widely from the average. Taking a maximum total absorption of 80 percent as the dividing line, the plants in the following mixtures absorbed most efficiently:

40 N × 10^{-6} KH_2PO_4 : 20 N × 10^{-6} $Ca(NO_3)_2$: 80 N × 10^{-6} $MgSO_4$,
20 " KH_2PO_4 : 20 " $Ca(NO_3)_2$: 100 " $MgSO_4$,
60 " KH_2PO_4 : 20 " $Ca(NO_3)_2$: 60 " $MgSO_4$,
20 " KH_2PO_4 : 60 " $Ca(NO_3)_2$: 60 " $MgSO_4$,
80 " KH_2PO_4 : 40 " $Ca(NO_3)_2$: 20 " $MgSO_4$,
40 " KH_2PO_4 : 60 " $Ca(NO_3)_2$: 40 " $MgSO_4$,
20 " KH_2PO_4 : 40 " $Ca(NO_3)_2$: 80 " $MgSO_4$,
60 " KH_2PO_4 : 40 " $Ca(NO_3)_2$: 40 " $MgSO_4$,
40 " KH_2PO_4 : 40 " $Ca(NO_3)_2$: 60 " $MgSO_4$,

Absorption increased in the order listed. As in the nitrate series the greatest absorption generally took place from solutions in which no one of the three salts greatly predominated. This fact is shown clearly in figure 2. All of the solutions most favorable to absorption occupy the center of the triangle. Nevertheless the wide range of variation in the composition of solutions almost equally favorable to absorption seemed to indicate that, as far as absorption is concerned,

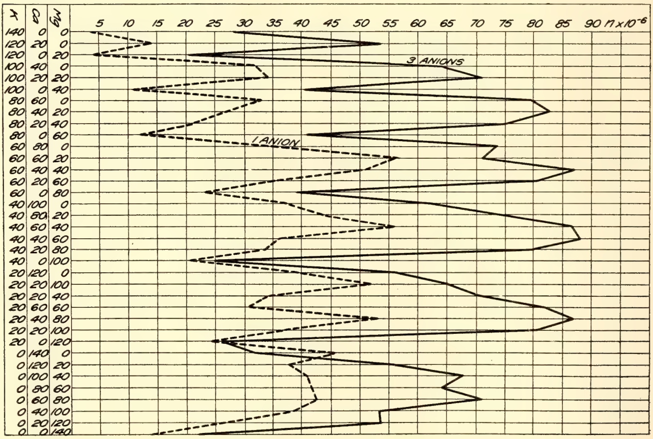

FIG. 3. Graph showing the absorption maxima of the nitrate mixtures (dotted line) and the mixtures with unlike anions (unbroken line).

roots may function efficiently in dilute solutions provided the concentration of no single ion is too greatly reduced. We seem to find here an argument for Liebig's "Law of the Minimum." When a sufficient concentration of an ion is present, the particular ratio of the different ions to one another is, within a rather wide range of variation, relatively immaterial. The significance of a full quota of anions as well as of cations stands out as the most striking feature of the second experiment.

On account of the different and varying temperature conditions under which the two experiments were carried out it would be unsafe to compare them too minutely. There can be no doubt however about the significance of the great difference between the absorption maxima in the two series. They are graphically represented in figure 3, and show the strikingly greater absorption which resulted from the presence of a full quota of anions.

CONCLUSIONS

1. In general, seedlings of *Lupinus albus* L. absorb more salts from mixtures of the nitrates of potassium, calcium and magnesium than from equally concentrated solutions containing only one or two of these nitrates.

2. The solutions of the 3 nitrates which were most favorable to absorption were much inferior to corresponding solutions in which three anions, $H_2PO_4^-$, NO_3^- and $SO_4^=$, were present. Under fairly comparable conditions the roots were able to absorb about half of the salts from the best solutions of the 3 nitrates and 85 percent from corresponding solutions with mixed anions.

3. In solutions of KNO_3, $Ca(NO_3)_2$ and $Mg(NO_3)_2$, as well as in solutions of KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$ the best absorption occurs when no single ion greatly predominates over the rest. Nevertheless, there is a wide range of variation in the proportion of different ions, within which range the roots absorb with almost equal efficiency.

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ON THE IDENTITY OF BLANCO'S SPECIES OF BAMBUSA

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In the year 1837 Blanco[1] described eight species of bamboo under the generic name Bambus, which, for the most part, have not been at all understood by later authors. It should be borne in mind that Blanco preserved no herbarium material, and that his descriptions, judged by modern standards, are very imperfect. Later authors having little or no botanical material from the Philippines, and having no special knowledge of field conditions in the Archipelago have usually merely enumerated Blanco's species with abbreviated descriptions compiled from the data given by Blanco. The single attempt previously made to reduce Blanco's species is notably inaccurate.

Steudel[2] includes all of Blanco's species with abbreviated descriptions under Blanco's names, except for *Bambusa mitis* Blanco which he changed to *Bambusa blancoi* Steud. Miquel[3] merely follows Steudel in his treatment of Blanco's species. Munro, in his monograph of the Bambuseae,[4] includes all of Blanco's species under "Bambusae minus notae" with abbreviated descriptions, accepting Steudel's *Bambusa blancoi* in place of *Bambusa mitis* Blanco. The next consideration of the Philippine species was by Fernandez-Villar,[5] who arbitrarily reduced all of Blanco's species to those of other authors, species that, with one exception, do not occur in the Philippines. With one exception all of F.-Villar's reductions are erroneous. In 1905 I was obliged to enumerate six of Blanco's species as unknown,[6] but am confident that the remaining two were correctly reduced. At this time very few specimens of Philippine bamboos had been collected in flower. As botanical exploration progressed, however, there was a rapid increase in fertile bamboo material, and in 1910 the accumulated collections were submitted to J. Sykes Gamble, Esq., for study. Mr. Gamble[7] published a critical enumeration of the species recognizing seven genera and twenty-five species. In a supplementary paper[8] he has added two genera and six species, making the total of known Philippine forms thirty-one, distributed in nine genera. Mr. Gamble, however, like other European botanists, had no detailed knowledge of the various forms as they occur in the field, and very wisely made no attempt to reduce Blanco's species; in fact he does not even enumerate them. Camus,[9] however, in his recent monograph of the group includes all of Gamble's species that were published before the year 1913, and at the same time includes all of Blanco's species, like Miquel, Steudel, and Munro, giving abbreviated descriptions from Blanco's data. Unlike Munro, however, he includes the species as valid ones, not as species of doubtful status. There is nothing to be gained in repeating these abbreviated descriptions of Blanco's species, for they are utterly inadequate as guides to the identification of the forms. Blanco's species should be either dropped entirely, or they should be interpreted with reference to all the data given by Blanco, growth form, habitat, distribution, time of flowering, uses, and native names. With a fair amount of field knowledge of the Philippines it is a comparatively easy matter for the local botanist to interpret most of Blanco's species, and to interpret them correctly. Without a knowledge of local conditions, the various types of vegetation, the native names and uses of plants, their relative abundance, distribution, time of flowering, etc., the task of correctly interpreting the species is a very difficult one. The case of the bamboos presents particular difficulties, as most species of bamboo rarely flower, and, without flowering specimens, attempts to classify the material meet with failure, especially as most of the Philippine bamboos are endemic. It is now possible correctly to interpret Blanco's species of bamboo, a task that would have been impossible before the Philippine collections were critically studied with reference to the entire Indo-Malayan bamboo flora. As was to be expected, most of Blanco's species are found to be the common and widely distributed ones in central Luzon at low altitudes, and all of them have been described by other authors under other names, some previous to Blanco, and some at a more recent date. In every case I am perfectly confident of the correctness of my interpretation of Blanco's species, and accordingly have not hesitated to accept his specific names where they prove to be valid. The eight species described by Blanco reduce to seven, two in the genus Bambusa, one in the genus Gigantochloa, and four in the genus Schizostachyum.

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[1] Fl. Filip. 268–272. 1837; ed. 2. 187–189. 1845; ed. 3, **3**: 333–338. 1879.

[2] Syn. Plant. Glum. **1**: 331. 1854.

[3] Fl. Ind. Batav. **3**: 420–21. 1855.

[4] Trans. Linn. Soc. **26**: 1–157. 1868.

[5] Novis. App. Fl. Filip. 323, 324. 1880.

[6] A Review of the Identifications of the Species described in Blanco's Flora de Filipinas. Govt. Lab. Publ. (Philip.) **27**: 1–132. 1905.

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[7] The Bamboos of the Philippine Islands. Philippine Journ. Sci. C. Bot. **5**: 267–281. 1910.

[8] Some Additional Bamboos of the Philippine Islands, op. cit. **8**: 203–206. 1913.

[9] Les Bambusées 1–215. *pl. 1–100.* 1913.

BAMBUSA Schreber

BAMBUSA BLUMEANA Schult. in Roem. & Schult. Syst. Veg. 7^2: 1343. 1830

Bambus pungens Blanco Fl. Filip. 270. 1837; Steud. Syn. Pl. Glum. 1: 331. 1854; Munro, Trans. Linn. Soc. **26**: 119. 1868.

Bambus arundo Blanco, op. cit., ed. 2, 188, ed. 3. **1**: 335. 1877, non Klein.

Bambusa arundinacea F.-Vill. Novis. App. 323. 1880, non Retz.

This species is widely distributed in the Philippines, occurring as a planted bamboo throughout the settled areas at low altitudes. It is certainly not a native of the Philippines, but a purposely introduced species and of prehistoric introduction. It is by far the most valuable building bamboo found in the Archipelago, and is very extensively utilized in all parts of the Philippines. The species originally described by Blanco as *Bambusa pungens* was changed by him in the second edition of his Flora de Filipinas to *Bambusa arundo. Bambusa arundinacea* F.-Vill. is merely a misidentification of *B. blumeana*, as *B. arundinacea* Retz. does not occur in the Philippines. *Bambusa blumeana* is remarkable for the very dense thicket of stiff, wiry, interlaced, much branched, very spiny branches that form an impenetrable thicket about the basal portions of the culms extending upward usually to a height of about two meters. *Arundarbor spinosa* Rumph. Herb. Amb. **4**: 14. *pl. 3* is unquestionably identical with *Bambusa blumeana*, but *Arundo agrestis* Lour. Fl. Cochinch. 72. 1790 (= *Bambusa agrestis* Poir. in Lam. Encycl. **7**: 708. 1808) is almost certainly a synonym of *Bambusa arundinacea* Retz. Loureiro cites Rumphius's *Arundarbor spinosa* under his *Arundo agrestis*, but the description is based on actual specimens from Cochinchina. Both *Bambusa blumeana* and *B. arundinacea* occur in Cochinchina, but Loureiro's description applies to the latter better than to the former.

BAMBUSA VULGARIS Schrad.; Wendl. Collect. Pl. **2**: 26. *pl. 47.* 1810

Bambus monogyna Blanco Fl. Filip. 268. 1837, ed. 2. 187. 1845, ed. 3. **1**: 333. 1877; Steud. Syn. Pl. Glum. **1**: 331. 1854; Munro, Trans. Linn. Soc. **26**: 119. 1868; Camus Bamb. 132. 1913.
Bambus mitis Blanco, op. cit. 271, 187, 336, non Poir.
Bambusa blancoi Steud. Syn. Pl. Glum. **1**: 331. 1854; Munro, Trans. Linn. Soc. **26**: 120. 1868; Camus, Bamb. 134. 1913.
Dendrocalamus strictus F.-Vill. Novis. App. 324. 1880, non Nees.
Dendrocalamus sericeus F.-Vill. l. c., non Munro.

This bamboo is widely distributed in the settled areas of the Philippines at low and medium altitudes, does not occur in the forested regions, and is usually, if not always, planted. It is not a native of the Philippines, but was undoubtedly purposely introduced in prehistoric times. *Bambusa monogyna* Blanco, for which he cites the Tagalog name *cauayang quiling* and *B. mitis* Blanco, for which he cites the Tagalog name *tiuanac*, are unquestionably the same species, which Blanco himself thought was possibly the case. The two native names are still in use in the vicinity of Manila exclusively for *Bambusa vulgaris* Schrad. *Bambusa blancoi* Steud. was merely a new name for *B. mitis* Blanco, non Poir., while *Dendrocalamus strictus* and *D. sericeus* are erroneous reductions of *Bambusa monogyna* and *B. mitis* on the part of F.-Villar; neither occurs in the Philippines.

GIGANTOCHLOA Kurz

Gigantochloa levis (Blanco) comb. nov.

Bambus levis Blanco Fl. Filip. 272. 1837, ed. 2. 189. 1845, ed. 3. **1**: 337. 1877; Steud. Syn. Pl. Glum. **1**: 331. 1854; Munro, Trans. Linn. Soc. **26**: 121. 1868; Camus, Bamb. 134. 1913.
Dendrocalamus flagellifer F.-Vill. Novis. App. 324. 1880, non Munro.
Gigantochloa scribneriana Merr. Philippine Journ. Sci. Suppl. **1**: 270. 1906.

This species is of wide distribution in the northern and central Philippines but is of local occurrence and is always planted, good evidence that it is not a native of the Archipelago, but like *Bambusa vulgaris* and *B. blumeana*, an introduced species. It is apparently very closely allied to and possibly identical with *Gigantochloa robusta*

Kurz, but at any rate Blanco's specific name is much the older. There is quite no doubt as to the identity of Blanco's *Bambusa levis*, for his description, while imperfect, applies only to the form I described as *Gigantochloa scribneriana* among all the Philippine species of bamboo.

SCHIZOSTACHYUM Nees

Schizostachyum diffusum (Blanco) comb. nov.

Bambus diffusa Blanco Fl. Filip. 269. 1837, ed. 2. 188. 1845, ed. 3. **1**: 334. 1877; Steud. Syn. Pl. Glum. **1**: 331. 1854; Munro, Trans. Linn. Soc. **26**: 118. 1868; Camus, Bamb. 131. 1913.
Schizostachyum acutiflorum Munro, Trans. Linn. Soc. **26**: 137. 1868; Gamble, Philippine Journ. Sci. C. Bot. **5**: 273. 1910; Camus, Bamb. 184. *pl. 95. f. A.* 1913.
Dinochloa diffusa Merr. Govt. Lab. Publ. (Philip.) **27**: 93. 1905.
Dinochloa major Pilger; Perkins, Fragm. Fl. Phil. 149. 1904.

This scandent bamboo is one of the most common and widely distributed sylvan species in the Philippines, and unlike most other Philippine bamboos it apparently flowers freely each year. Consequently it is much better represented in collections than any other Philippine form. It is distinctly variable, which leads me to suspect that *Schizostachyum dielsianum* (Pilg.) Merr. may not really be specifically distinct. Munro suggested that *Bambusa diffusa* Blanco was merely a variety of his *Schizostachyum acutiflorum*, while F.-Villar definitely made the reduction. I am now confident that *Bambusa diffusa* Blanco and *Schizostachyum acutiflorum* Munro are identical. Philippine material is now available in which the leaves are somewhat pubescent on the lower surface, thus agreeing with Blanco's description "pelosas por debajo," material that otherwise cannot be distinguished from typical *Schizostachyum acutiflorum* Munro. Blanco's description otherwise as to habit, habitat time of flowering, native names, and uses closely applies. The oldest specific name is here adopted.

Schizostachyum lima (Blanco) comb. nov.

Bambus lima Blanco, Fl. Filip. 271. 1837, ed. 2. 189. 1845, ed. 3. **1**: 336. 1877; Steud. Syn. Pl. Glum. **1**: 331. 1854; Munro, Trans. Linn. Soc. **26**: 121. 1868; Camus, Bamb. 134. 1913.
Bambusa longinodis F.-Vill. Novis. App. 323. 1880, non Miq.

Schizostachyum hallieri Gamble, Philippine Journ. Sci. C. Bot. **5**: 274. 1910.

The identity of this species is unquestionable, as it is the only bamboo known from the Philippines with very long internodes, a character expressly indicated by Blanco. Moreover it is the species invariably and consistently known to the Tagalogs as *anos*, the native name cited by Blanco. Gamble's objection to this identification of Blanco's *Bambusa lima*[10] was based on an erroneous translation of Blanco's description by Munro, whose description reads "foliis . . . angustis," while Blanco's original description reads "hojas . . . anchas," that is wide, not narrow leaves.

Schizostachyum lumampao (Blanco) comb. nov.

Bambus lumampao Blanco Fl. Filip. 272. 1837, ed. 2. 189. 1845, ed. 3. **1**: 338. 1877; Steud. Syn. Pl. Glum. **1**: 331. 1854; Munro, Trans. Linn. Soc. **26**: 118. 1868; Camus, Bamb. 132. 1913.

Dendrocalamus membranaceus F.-Vill. Novis. App. 324. 1880, non Munro.

Schizostachyum mucronatum Hack. Philippine Journ. Sci. C. Bot. **3**: 169. 1908; Gamble op. cit. **5**: 276. 1910; Camus, Bamb. 175. 1913.

There is quite no doubt as to the correctness of this interpretation of Blanco's *Bambusa lumampao*. While the description is short and imperfect, it applies entirely to *Schizostachyum mucronatum*. This bamboo is exceedingly abundant in the provinces near Manila, is gregarious over large areas, and quickly occupies deserted clearings on the hills and lower slopes of mountains to the practical exclusion of other forms of vegetation. While now more commonly known to the natives as *boho* or *caña boho*, it is in some regions still known as *lumampao*, and in others as *bocaui*, the native names cited by Blanco. As Blanco states the culms are about as thick as one's wrist, and the canes are still brought to Manila for certain purposes, notably for the woven building material known as *saule*, used for making walls, partitions, and ceilings in light construction houses.

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[10] Philippine Journ. Sci. C. Bot. **5**: 275. 1910.

Schizostachyum textorium (Blanco) comb. nov.

Bambus textoria Blanco, Fl. Filip. 270. 1837, ed. 2. 189. 1845, ed. 3. 1: 335. 1877; Steud. Syn. Pl. Glum. 1: 331. 1854; Munro, Trans. Linn. Soc. **26**: 122. 1868; Camus, Bamb. 135. 1913.

Gigantochloa atter F.-Vill. Novis. App. 323. 1880, non Kurz.

Schizostachyum merrillii Gamble, Philippine Journ. Sci. C. Bot. **5**: 278. 1910.

Blanco's description is very short and imperfect, and he saw no flowering specimens. He cites the Tagalog name *calbang*, and states that the species is common in some, but not in all forests. For a number of years attempts to locate a bamboo known to the natives as *calbang* failed, but in the year 1914 a characteristic species was found to be commonly known by this name in Batangas Province, Luzon, a region from which Blanco received much of his botanical material. This Batangas *calbang* agrees with Blanco's description, so far as the description goes, and is identical with *Schizostachyum merrillii* Gamble. The oldest specific name is here adopted.

THE REGION OF GREATEST STEM THICKNESS IN RAPHIDOPHORA

Frank C. Gates

To one accustomed to expect the greatest diameter in the oldest part of the stem, several tropical vines are interesting exceptions. Conspicuous in this respect are the araceous genera, Raphidophora and Epipremnum. Vines of *Raphidophora merrillii* Engl., growing at Los Baños, Philippine Islands, were chosen for a series of measurements to present the anomaly more clearly. Measurement of the thickness of the stem was taken at 5 cm. intervals beginning at the tip. Of the 37 plants employed the measurements of 7 are given in the accompanying table.

In each case it will be noted that the oldest part of the stem is not as thick as near the tip. In an extreme case the stem was more than seven times as thick near the tip as it was in the oldest region. In all cases the oldest region had the smallest diameter, which in the plants measured was 0.15 cm. The greatest thickness of any stem was 3.2 cm. At the tip, where the developing tissues have not yet reached their full size, the diameter of the stem is somewhat less than the maximum thickness, which however occurs within 15 cm. of the tip.

Further analysis makes this condition seem less anomalous. As the plant becomes larger the new leaves are larger, carry on more photosynthesis and thus furnish more food. This food is carried only very short distances in the stem. Absorption by clingfast roots, which occur along the whole stem, adds materially to the water supplied through the main root. Owing to the large number of side roots, the main root performs proportionally less and less work, yet is of value as it is usually rooted in the ground, where there is a permanent water supply. The side roots obtain their water supply from the water soaked up by the debris accumulated between them and the tree trunk upon which the vine is growing. This is entirely ample during the rainy season. It frequently happens that side roots may grow out and down to the ground. This further reduces the demands upon the main root. Usually the old stem does not die off, although it may shrivel somewhat. If however, the old stem is cut off, the plant continues to develop, obtaining its water supply entirely from side roots.

The youngest part of the stem is the most fleshy. As it becomes older it becomes less fleshy, but retains approximately its maximum diameter. As the whole plant becomes larger the increasing in thickness of the newly forming stem continues, but at a much slower rate, so that a stem of Raphidophora 4 cm. in thickness is very unusual.

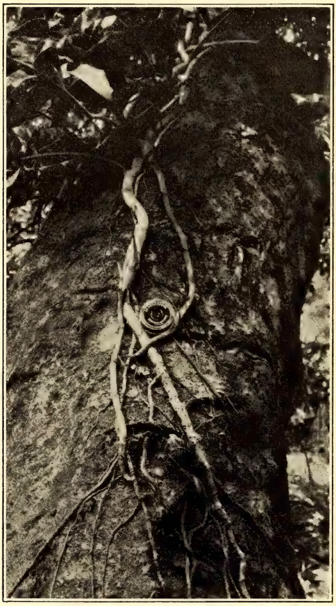

FIG. 1. Vines of Raphidophora merrillii Engl. growing on a leaning mango tree, illustrating the fact that the stem is progressively thicker in its younger portions. Los Baños, P. I., Nov. 3, 1913.

DOUGLAS LAKE, MICHIGAN

Table of Stem Thickness of Seven Plants of *Raphidophora merrillii* at 5 Cm. Intervals from the Tip

From Tip	1	2	3	4	5	6	7
....	....	....	2.4	1.6	....	.3	1.5 cm.
5 cm.	.85	.85	2.8	1.8	.90	.3	2.0
10	1.00	1.10	2.6	2.4	.85	.6	2.8
15	.95	1.10	2.5	2.7	.80	1.0	2.6
20	.95	.80	2.1	3.0	.75	.9	2.4
25	.95	.95	1.8	2.9	.73	.9	2.3
30	.90	.80	1.7	2.7	.70	.8	2.2
35	.90	.65	1.6	2.6	.67	.8	2.1
40	.85	.60	1.5	2.7	.64	.7	2.0
45	.75	.60	1.3	2.6	.60	.7	1.8
50	.75	.55	1.3	2.5	.60	.7	1.8
55	.65	.50	1.1	2.4	.55	.7	1.5
60	.60	....	1.1	2.5	.54	.6	1.3
65	.60	....	1.1	2.4	.52	.5	1.2
70	.50	....	1.0	2.4	.50	....	1.0
75	....	....	1.1	2.3	.47	....	1.0
80	....	....	0.9	1.9	.45	....	.9
85	....	....	1.0	1.8	.43	....	.9
90	....	....	0.8	1.5	.40	....	.8
95	....	....	0.8	1.2	.39	....	.7
100	....	....	0.8	1.2	.38	....	....
105	....	....	0.7	1.0	.35	....	....
110	....	....	0.8	1.0	.30	....	....
115	....	....	....	.95	.25	....	....
120	....	....	....	.80	.25	....	....
125	....	....	....	.85	.20	....	....
130	....	....	....	.80	.15	....	....
135	....	....	....	.70	....	....	....
140	....	....	....	.70	....	....	....
145	....	....	....	.60	....	....	....
150	....	....	....	.60	....	....	....
155	....	....	....	.60	....	....	....
160	....	....	....	.50	....	....	....
165	....	....	....	.50	....	....	....
170	....	....	....	.45	....	....	....
175	....	....	....	.40	....	....	....

THE MECHANISM OF MOVEMENT AND THE DURATION OF THE EFFECT OF STIMULATION IN THE LEAVES OF DIONAEA[1]

William H. Brown

Introduction

Charles Darwin (1875) appears to have been the first to investigate the mechanism of leaf closure in Dionaea. He marked the upper surface of the leaf with ink-dots and found that the distances between these decreased slightly as the leaf closed. From this he concluded that closure is due to the active contraction of the upper surface of the leaf. De Candolle, as a result of his morphological studies of Dionaea, advanced the idea that the opening and closing movements of the leaf are due to changes in the turgescence of the parenchyma of the dorsal region. Batalin (1877) concluded that movement is here accompanied by a small amount of actual growth. Munk (1876) expressed the opinion that the closure of the leaves is mainly due to the contraction of the upper surface but added that there is also an expansion of the lower surface. This writer supposed that water passes from the cells of the upper to those of the lower region. Macfarlane (1902) believed that there are structures in the leaves of Dionaea which resemble animal muscles. The prevailing opinion thus seems to have ascribed the closure of Dionaea leaves to the contraction of the dorsal region of the leaf.

The experiments here reported were carried out at the Laboratory of Plant Physiology of the Johns Hopkins University, and the writer is indebted to Prof. B. E. Livingston for valuable assistance in the experimentation and for editorial help in connection with this paper. The plants used were very kindly supplied by Dr. W. D. Hoyt. The fact that much of the literature dealing with plant movements is not available at this Bureau has rendered the above discussion of the literature necessarily very incomplete.

Mechanism of Stimulation Movements

The leaves of Dionaea show two distinct types of closing movements. In the first of these, if the leaf is stimulated and the stimulating object is then withdrawn the two lobes approach each other rapidly, the leaf returning to its original condition only after several hours, usually by the following day. When the closure occurs in this way the marginal bristles of the two lobes become interlaced, while the lobes themselves bulge out widely from each other with their ventral surfaces convex. In the second type of movement, when the leaf closes over an insect, the two lobes approach each other in such a way that the ventral surfaces often become concave. This brings the upper surfaces into closer contact with the insect and leaves a smaller opening between the two lobes than is the case with the first type of closure. In the present discussion only the first type of movement will be considered.

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[1] Botanical contribution from the Johns Hopkins University, No. 48.

In order to determine what region of the leaf is most active in causing the closing movement, both the dorsal and ventral surfaces were similarly marked with rows of dots made with India ink and running both parallel to and perpendicular to the midrib. The distances between the dots having been microscopically measured, the leaves were stimulated and the measurements were repeated after closing and again after reopening. In order to measure these distances on the dorsal surface of one lobe, it was necessary to remove part of the opposite lobe, which was accomplished by removing an oblong piece with a sharp scalpel, several days being allowed for the leaf to recover before the beginning of the experiment itself. Such measurements were made on both young and mature leaves of various sizes, but always with similar results.

In Table I. are given the measurements made on the ventral surfaces of five different leaves; the numerals of the experiment numbers refer to the leaf and the letters refer to the successive spaces between dots, from the midrib outward to the margin. The values themselves are merely relative. In the case of No. 1 the leaf was caused to close three successive times and was allowed to reopen after each closure. From these measurements it appears at once that the distances between the transversely arranged dots on the ventral surface increase considerably when the leaf closes and change comparatively little during reopening.

To study the transverse expansion of the lower leaf surface thus indicated as accompanying closure, the percentage of this expansion was calculated for each distance, on the basis of the corresponding measurement obtained when the leaf was in the original open condition. The percentages thus derived are presented, for nine different leaves, in Table II, which includes the data for the leaves referred to in Table I with the corresponding experiment numbers.

TABLE I.

Comparative Measurements of Distances Between Adjacent Dots on the Lower Surface of Dionaea Leaves Before and After the Closing Movement, the Dots Arranged in a Line Transverse to the Midrib

Experiment No.		Leaf Open	Leaf Closed	Leaf Open	Leaf Closed	Leaf Open	Leaf Closed
1	*a*	20.0	22.0	20.0	23.0	23.0	23.0
	b	23.0	23.5	23.5	26.0	27.0	28.0
	c	23.0	29.0	30.0	34.0	34.0	35.0
	d	30.0	34.0	35.0	36.0	36.0	38.0
	e	....	19.0	19.0	20.0	21.0	21.5
2	*a*	21.0	22.0	....	....	....	....
	b	26.0	28.0	....	....	....	....
	c	37.0	40.5	....	....	....	....
	d	25.0	26.5	....	....	....	....
	e	26.0	27.0	....	....	....	....
3	*a*	23.0	23.0	24.0	....	....	....
	b	31.0	32.0	33.0	....	....	....
	c	26.0	29.0	29.0	....	....	....
	d	21.0	22.0	23.0	....	....	....
4	*a*	25.0	27.0	....	....	....	....
	b	17.0	18.0	....	....	....	....
	c	17.0	19.0	....	....	....	....
	d	16.0	17.5	....	....	....	....

From the data of Table II it appears that the lower surface of the leaf lobe expands transversely during the process of closing, the average amount of this expansion for the leaves tested being 6.7 percent of the original distance from the first to the last dot.

The results of similar measurements of the distances between dots placed in rows parallel to the midrib gave similar results, and it thus appears that the area of the whole lower surface increases in extent during closure.

Open leaves are usually slightly curved so that the dorsal surface is somewhat concave and the ventral convex. During the process of closing, with this type of movement, as has been remarked, this curvature becomes much more pronounced. Since the measurements did not give the distances between the dots along the curved surface, but only the rectilinear distances, the amounts of expansion shown in Table I are less than those actually occurring. The distance measured was always the chord of the arc of curvature of the leaf surface.

TABLE II

Percentage of Increase with Leaf Closure, in Distances Between Adjacent Dots on the Lower Surface of Dionaea Leaves, the Dots Arranged in a Line Transverse to the Midrib

Experiment No.	Expansion, 1st Closure	Expansion, 2d Closure	Expansion, 3d Closure
1 a	10.0	15.0	0.0
b	2.2	10.6	3.7
c	26.1	13.3	2.9
d	13.3	2.8	5.5
e	....	5.3	2.5
Between extreme dots	13.0	9.0	3.2

Experiment No.	Expansion	Experiment No.	Expansion
	Percent		*Percent*
2 a	4.8	3 a	0.0
b	7.7	b	3.2
c	9.5	c	11.5
d	6.0	d	4.8
e	3.8		
Between extreme dots	6.7	Between extreme dots	4.9
4 a	8.0	5 a	12.5
b	5.9	b	15.8
c	11.8	c	11.6
d	9.4	d	10.1
		e	0.0
Between extreme dots	8.7	Between extreme dots	10.0
6 a	1.8	7 a	3.3
b	4.8	b	8.4
c	3.8	c	3.7
d	2.6	d	1.5
		e	1.2
Between extreme dots	3.3	Between extreme dots	3.9
8 a	0.0	9 a	3.4
b	30.0	b	13.6
c	3.3	c	2.8
Between extreme dots	5.7	Between extreme dots	5.7

The results of measurements similar to those just described, made on the upper leaf surface, are given in Table III, which also gives percentages of decrease in the distances between the dots during closing and of increase during opening, as compared to the original distances.

Table III

Comparative Measurements of Distance Between Adjacent Dots on the Upper Surface of Dionaea Leaves Before and After Closing and After Reopening, the Dots Arranged in a Line Transverse to the Midrib, Together with Percentage of Shrinkage During Closure and of Expansion During Reopening

Experiment No.		Distances, Leaf Open	Distances, Leaf Closed	Shrinkage During Closure	Distances, Leaf Reopened	Expansion During Reopening
				Percent		*Percent*
1	*a*	19.0	19.0	0.0	20.0	5.3
	b	20.0	19.7	1.5	22.0	11.7
	c	16.0	15.8	1.3	17.0	7.6
	d	11.0	10.5	4.5	11.5	9.5
Between extreme dots		....	....	1.5	....	9.3
2	*a*	24.0	24.0	0.0	....	....
	b	27.0	26.8	0.7	....	....
	c	20.0	19.7	1.5	....	....
	d	15.0	15.0	0.0	....	....
Between extreme dots		....	....	0.6	....	....
3	*a*	20.0	19.5	2.5	....	....
4	*a*	....	13.5	....	15.0	11.1
	b	....	19.0	....	20.0	5.3
	c	....	25.0	....	27.0	8.0
	d	....	21.0	....	24.0	14.3
Between extreme dots		....	....	....	....	9.6

From the data of Table III it appears that these dots on the upper surface of the leaf approach each other to a slight degree during the closing process. The average decrease thus occurring in the distances between extreme dots of these transverse rows is 1.5 percent of the original distance.

This apparent transverse shrinkage of the upper surface may be explained by the error of measurement just pointed out, due to curvature; the bending of the lobe which accompanies closure should bring the dots on the upper surface closer together without any actual change in the extent of this surface. To test this suggestion, a paper marked with dots at measured distances apart was bent so as to have a form similar to that of a curved leaf lobe, after which the distances between the dots were again measured. In this case the dots approached each other to a greater extent than did those on the dorsal surfaces of the leaves during closure. This seems to indicate that if there is any change in the area of the upper surface during closure it is probably in the direction of an increase rather than in that of a decrease. Although this point is uncertain, it is still quite clear that if any transverse shrinkage occurs in the area of the upper leaf surface this must be practically negligible when compared with the expansion that has been shown for the lower surface. It is thus strongly suggested that the movement of closing is largely due to the increase in volume of tissues lying near the lower leaf surface, although, as will be pointed out later, there appears also to occur a decrease in the turgor of the tissues near the upper surface, which probably also has a contributory effect. This conclusion is contrary to the prevailing opinion in this connection, that leaf closure is due to the contraction of the dorsal tissues.

The reopening of the leaf after it has closed, on the other hand, seems to be due to expansion of the upper layers of cells, for during this process the area of the lower surface changes only slightly while that of the upper enlarges considerably, the measurements here showing an average increase of 9.4 percent.

TABLE IV

Comparative Measurements of Distances Between Adjacent Dots on the Lower Surface of Dionaea Leaves just Before Closing, just After Closing, and 1, 2, and 6 hours After Closing, the Dots Arranged in a Line Transverse to the Midrib

Experiment No.		Just Before Closing	Just After Closing	1 Hour After Closing	2 Hours After Closing	6 Hours After Closing
1	*a*..........	12.0	12.0	12.5	12.5	....
	b..........	5.0	6.5	7.0	6.5	....
	c..........	16.5	17.0	17.0	16.5	....
2	*a*..........	14.5	15.0	15.0	15.0	....
	b..........	11.0	12.5	12.5	12.0	....
	c..........	18.0	18.5	19.0	19.0	....
3	*a*..........	21.0	22.0	22.0	22.5	22.5
	b..........	26.0	28.0	28.0	28.0	28.0
	c..........	37.0	40.5	40.0	40.5	40.5
	d..........	25.0	26.5	26.5	25.5	25.5
	e..........	26.0	27.0	26.5	26.5	26.5

Examination of the data for experiments 1 and 2, given in Table I, indicates that the increase in area of the lower surface during closure is practically permanent and that this enlargement persists after the leaf reopens. That this increase generally remains fairly constant during the period between closing and reopening is shown by the data given in Table IV, in which are recorded the results of hourly measurements made during this period.

The average increase in the distance between the first and last dot on the lower leaf surface, for the entire period of closing and reopening, may be calculated by adding the average percentage increase during closure (6.7 percent) to the average percentage during opening (1.4 percent), the result being 8.1 percent. In the case of the upper leaf surface, the average percentage of shrinkage during closure (1.5 percent) is to be subtracted from the average percentage of expansion during opening (9.4 percent) leaving 7.9 percent as the total percentage of increase in the transverse direction for the upper surface. These two values are very nearly alike and their average, which is 8.0 percent, may be taken to represent the transverse enlargement of the lobe during the entire period of closing and reopening.

It is worthy of note that the average transverse shrinkage of the upper surface during closure (1.5 percent) is nearly equal to the average expansion of the lower surface during opening (1.4 percent). As has been seen, the former of these apparent changes is probably to be explained as due to an error in measurement, resulting from the curvature of the leaf lobe when closed, and it seems equally probable that the measurement of the lower surface during opening is subject to a similar error, but of opposite direction resulting from the straightening of the leaf lobe, which makes the dots appear farther apart in rectilinear distance. It is therefore not unlikely that all of the apparent expansion of the lower surface during opening is attributable to this error.

It remains to enquire whether the rate of transverse enlargement of these leaf lobes is greater during the period of closing and opening than at other times. As shown above, this enlargement amounts to about 8.0 percent. To answer this question three leaves of different ages were selected and one lobe of each was marked on the lower surface, with a row of ink dots reaching from the midrib to the margin as in the previous cases, the distances between the dots being then measured at intervals of from 1 to 8 days. The results of these measurements are given in Table V.

The last column of Table V presents the total amount of enlargement for the entire period of observation, on the basis of the original measurements. The first of the experiments here referred to continued 18 days, and the total transverse enlargement, from the first to the last dot, was only 3.1 percent for this entire period, which is much less than the enlargement in the same direction shown by the lower surface of stimulated leaves for the much shorter period of closing and reopening. The second experiment lasted seven days and the total expansion recorded was 1.4 percent. The third experiment also continued seven days, but showed a greater amount of total enlargement than did either of the other two, this being 9.6 percent. If the percentage of total transverse enlargement of the ventral surface of the leaf lobe be divided by the number of days in each case, the

TABLE V

Comparative Measurements of Distances Between Adjacent Dots on the Lower Surface of Unstimulated Dionaea Leaves, the Dots Arranged in a Line Transverse to the Midrib, to Show Rates of Transverse Enlargement

Experiment No.		At Beginning of Experiment	After 1 Day	After 2 Days	After 3 Days	After 4 Days	After 6 Days	After 7 Days	After 8 Days	After 10 Days	After 18 Days	Total Enlargement
												Percent
1	*a*	22.0	22.0	23.0	23.0	....	22.0	....	22.0	22.0	22.0	0.0
	b	25.0	25.0	25.0	26.0	....	26.0	....	26.0	26.0	26.0	4.0
	c	30.5	30.5	30.0	30.5	....	31.0	....	31.0	31.0	31.5	3.3
	d	33.0	33.5	33.3	33.0	....	33.0	....	33.0	33.0	34.5	4.5
	e	18.0	18.0	18.0	18.0	....	18.5	....	18.0	18.0	18.5	2.8
Between extreme dots		......	....	....	....	....	....	....	....	....	....	3.1
2	*a*	27.0	....	27.0	....	27.0	....	27.0	....	....	....	0.0
	b	33.5	....	33.5	....	34.0	....	34.0	....	....	....	1.5
	c	26.0	....	26.0	....	27.0	....	27.0	....	....	....	3.9
	d	19.0	....	19.0	....	19.0	....	19.0	....	....	....	0.0
Between extreme dots		......	....	....	....	....	....	....	....	....	....	1.4
3	*a*	13.5	....	13.5	....	15.0	....	15.0	....	....	....	11.1
	b	19.0	....	19.0	....	20.0	....	20.0	....	....	....	5.2
	c	25.0	....	25.0	....	26.5	....	27.0	....	....	....	8.0
	d	21.0	....	21.0	....	23.0	....	24.0	....	....	....	14.3
Between extreme dots		......	....	....	....	....	....	....	....	....	....	9.6

average daily rates for the three cases prove to be: 0.17, 0.20, and 1.36 percent, respectively. The highest of these average daily rates is only 43.7 percent of the smallest corresponding total enlargement recorded for the period of closing and opening in the case of stimulated leaves. The average daily rate of increase in the distance between the extreme dots on the lower surface in the case of unstimulated leaves is 0.58 percent, while the average corresponding enlargement in the case of stimulated leaves, during closure alone is 6.73 percent. Thus, the transverse expansion of the ventral surface during closure of stimulated leaves is 11.6 times as great as is the average daily rate of transverse enlargement shown by the lower surfaces of unstimulated leaves. From these figures it is evident that the stimulation movements are accompanied by a greatly accelerated rate of transverse enlargment.

Accelerated activity is, in general, accompanied by depletion of previously accumulated material, by increased formation of the products of cell activity, and sometimes even by the formation of products not otherwise produced. It is therefore suggested that, if closure of these Dionaea leaves is accompanied by accelerated growth, the response should be less vigorous after successive closures, and the results of experiment 1, as shown in Tables I and II, are in agreement with this suggestion. In this experiment the distance between the first and last dot increased 13.0 percent during the first closure, 9.0 percent during the second, and only 3.2 percent during the third response. Moreover, after this last closure the leaf opened very slowly and the process required a number of days, the extreme slowness of the opening movement rendering it practically impossible to decide just when the opening was complete. Also, it has been generally found in this study that plants become sickly and die when the leaves are repeatedly stimulated at short intervals.

The idea is at once suggested that we are here dealing with something closely related to the condition of fatigue, which has been clearly demonstrated for certain other motile responses in plants. For example, the stigma lobes of *Martynia* (Brown, 1913) respond to contact stimulation by a rapid drawing together, which is followed by a return to the usual position, the latter process requiring from 8 to 40 minutes. If these stigmas are repeatedly stimulated, each new stimulus being applied as soon as the lobes have regained their usual position, the period required for opening may become shorter after the first and second response, but it then becomes longer and longer as stimulation is repeated, until the stigmas at length fail to respond to contact stimulation at all. Here it is not clear whether the decrease in rate of movement is due to an accumulation of toxic substances, as in the case of animal muscle, or to the using up of available material, but the latter supposition seems to be the more probable of the two.

In the case of Dionaea it also appears probable that the decreased activity observed with repeated stimulation may be due to depletion of previously accumulated materials, for considerable material must be expended in the excessive growth that follows stimulation. Such an enlargement might occur on account of either one of three possible causes, or on account of two or more acting together. (1) A sudden increase in the osmotic pressure of the cells that expand might stretch the cell walls and lead to permanent enlargement, without any preliminary change in the walls themselves. (2) The osmotic pressure of the cells in question might remain the same, but the extensibility of their cell walls might be increased (or their tendency to contract decreased) so that the same pressure as was previously effective only to hold them in equilibrium might now produce enlargement. This hypothesis seems very improbable. (3) Finally, the osmotic attraction for water exerted by the cells near the upper surface might be decreased, so as to allow water to pass from these cells into those near the lower surface, thus allowing an expansion of the latter due to their original osmotic pressure. In any of these three cases the enlargement of the tissues on the lower side of the lobe must be concomitant with the passage of water into these cells due to their osmotic activity.

Evidence will be adduced below showing that stimulation is followed by a decrease in the osmotic pressure of the cells of the upper layers, which would allow the passage of water out of these cells into those of the lower region, but it seems hardly probable that sufficient water to cause the observed changes may thus enter the lower cells unless one of the other possibilities just mentioned also becomes effective; it is to be supposed that these lower cells have attained equilibrium, as far as water is concerned, under the previously existing conditions of their osmotic pressure and of the elasticity of their walls. If the closing movement of Dionaea leaves occurred only at times of the day when incipient drying of the lower leaf tissues [Livingston and Brown (1912)] might be postulated, then the enlargement of these cells might occur because of the release of a large amount of water from the cells of the opposite side, but these leaves are capable of vigorous movement at all hours of the day, so that this supposition cannot be upheld.

If the closing movement were due to a stretching of the cell walls of the lower region, later rendered permanent by growth, then leaves killed just after closure might perhaps be made to reopen by replacing the water in the cells by some liquid in which their osmotically active solutes were insoluble. By thus precipitating these solutes the osmotic pressure of the cells would be removed and the leaves might resume their original form. Returning such leaves to water should then cause them to close again, providing the protoplasmic membranes had not been too greatly altered and providing the re-entering water might replace the other liquid and again bring the solutes into aqueous solution. In such a case the original osmotic pressure would be restored to all cells and those of the ventral region should again become stretched as at first. To test this possibility a number of leaves (some open, some just closed by stimulation, and some closed for a half-hour or longer) were killed in boiling water and then passed through alcohol to xylene. Since sugars are practically insoluble in xylene, the replacement of the water of the cells by this liquid should result in a precipitation of sugars, which may be supposed to be of prime importance in producing the usual osmotic pressure. The leaves killed just after closure reopened in xylene, while those that were open and those that had been closed for some time when killed showed no alteration. All of the leaves were then returned through alcohol to water, which resulted in re-closure of those that had opened in xylene, while the others still remained unaltered. In some cases such transfers from water to xylene and back again were repeated a number of times with the same leaf, and the results were always like those just described. As might be expected the distances between adjacent ink-dots on the lower surfaces of leaves, showing movement decreased when the leaves were changed from water to xylene and increased when the reverse transfer was made. The comparative measurements from such a transfer of a leaf killed just after closing, from water to xylene and back to water, are given in Table VI.

TABLE VI

Comparative Measurements of Distances Between Adjacent Dots on the Lower Surface of Dionaea Leaves Killed Just After Closing, as the Leaves were Transferred From Water Through Alcohol to Xylene and Back to Water, the Dots Arranged in a Line Transverse to the Midrib

Experiment No.		Leaf in Water	Leaf in Alcohol	Leaf in Xylene	Leaf in Alcohol	Leaf in Water
I	*a*.	42	42	37	42	42
	b.	28	27	26	27	28
	c.	52	50	46	50	52

It appears that the only way by which transfer from water to xylene may produce opening of the leaf, as above described, must be through the removal of the osmotic pressure in all of the cells, and these experiments seem to show that the stimulation closure that had occurred just before the experiment began was due to a stretching, by osmotic pressure from within, of the cell walls of the ventral portion of the leaf. It thus appears that the movement of closure is due to stretching of these cell walls rather than by ordinary growth. The enlargement thus effected is soon fixed by permanent growth changes, however, as is shown by the fact that the transfer from water to xylene does not affect the form of the leaves if these have been closed for a half-hour before being killed. Since the tests involving measurements of the changes in the surface dimensions of normal leaves as these close and reopen agree with the experiment just described, it seems clear that stimulation closure is not due to an active contraction of the tissues near the dorsal surface, but is due to osmotic expansion of the cells in the ventral region.

The supposition that the closing response in Dionaea leaves may be due to alterations in the permeability of the protoplasmic membranes seems to be excluded by the experiments described above. Enlargement of the cells in the ventral region must be preceded and accompanied by entrance of water into these cells, probably from those of the dorsal region. Changes in permeability might account for the passage of water out of the latter cells although as Pfeffer (1906) points out this would necessitate the movement of dissolved substances along with the water. Changes in permeability can not, however, explain the passage of water into the cells of the ventral region of the leaf, and it is this movement of water which appears to be the first condition necessary for stimulation closure.

A study of the cell contents in stimulated and unstimulated leaves of Dionaea brought out some interesting and apparently important facts. Forty-eight apparently normal and sensitive leaves were killed in boiling water, some open, others just after closing and still others fifteen minutes or more after closing, and all were then examined for starch. Those killed while open showed very little or no starch in any of their cells, but when starch occurred most of it was in the upper epidermis and all of it was in the upper region of the leaf, between the veins and the dorsal surface. Leaves killed just after closing gave similar results. Those that had been closed for fifteen minutes or more at the time of killing showed all of the cells between the veins and the dorsal surface packed with starch, while the cells between the veins and the ventral surface contained no starch. The formation of starch thus indicated can not be considered as responsible for closure, since it did not occur until after the leaf had been closed for some time, but this starch formation may have been connected in some way with the same conditions as those that led to closure.

According to Pfeffer (1900, p. 326), Böhm was able to produce a deposition of starch by plasmolyzing cells with potassium nitrate solution. This suggests that the formation of starch occurring soon after closure in the cells of the dorsal region of Dionaea leaves may be caused by a pronounced extraction of water from these cells, such an extraction being brought about through a greatly increased absorptive power of the cells of the ventral region. It seems unlikely, however, that the osmotic concentration of the solution in the latter cells may become great enough to withdraw water from the cells of the dorsal region in sufficient amount to bring about the deposition of starch in the quantities observed.

It seems more probable that the sugar in the cells of the dorsal region of the leaf becomes less active osmotically as a response to stimulation, perhaps by being changed into some substance intermediate between sugar and starch, and if this occurs it should allow a movement of water out of these cells into the cells of the ventral region. Whether or not this sort of removal of sugar from solution in the dorsal cells does actually occur as a concomitant of leaf closure, it appears highly probable that there occurs, with stimulation, an increase in the osmotic attraction for water in the cell sap of the ventral cells. As has been mentioned above, it seems improbable that a sufficient amount of water to cause the observed stretching may pass into the cells of the ventral region unless this movement is preceded by an increase in the osmotic attraction for water exerted by the latter cells. That the causal change may be an alteration in the cell walls of the ventral region appears highly improbable, as has been noted.

In connection with the changes in the starch content of the tissues of these leaves, it may be mentioned that leaves that failed to respond to stimulation showed peculiarities in their starch content. There appeared to be two classes of leaves that did not respond to stimulation in these studies. Those of the first class were exceptionally thick and the lobes were usually slightly reflexed. While in this condition, which did not appear to be connected with their age, they did not respond to mechanical stimulation but they became sensitive later. During the apparently insensitive phase all of the cells contained considerable starch. Leaves of the second class were exceptionally thin, never contained starch either before or after stimulation and never showed any response. Such leaves were characteristic of certain plants, and were not observed to alter in the respects mentioned as they became older. The large amount of starch found in the first class of insensitive leaves, suggesting high osmotic pressure of their cells, and the entire absence of starch in leaves of the other class may perhaps be connected with the failure of these leaves to respond to mechanical stimulation.

The curvature of the primary pulvini of Mimosa, according to Pfeffer (1906), while aided by the force of gravitation acting upon the leaf segments, is mainly produced by an active contraction of the cell walls of the lower region and by compression exerted by the cells of the upper region, the latter remaining turgid after stimulation while the lower cells become flaccid. This flaccidity is brought about by loss of turgor due to the passage of water out of the cells into the intercellular spaces of the shrinking region of the pulvinus. Brown (1912) has shown that curvature may be produced in killed pulvini of Mimosa leaflets, as in the leaves of Dionaea, by transferring them from water through alcohol to xylene. In Mimosa, however, no movement is produced if the curvature has been completed before the pulvini are killed. If curvature has not been completed at the time of killing, transfer to xylene results in the completion of the curvature, and a return from xylene to water causes these leaflets to resume the position which they had when killed. Transfer from water to xylene must reduce the osmotic pressure in all the cells of the pulvini, and the movement of the dead organs seems, therefore, to have been due to shrinkage of the cells of the concave region while those of the convex region remained more rigid.

These experiments appear to be in agreement with Pfeffer's results, and they emphasize an apparent difference between Mimosa and Dionaea. In Dionaea, only leaves killed just after closing show opening movement when transferred from water to xylene; such leaves open by reason of decreased osmotic pressure in the cells of the convex region, this pressure having previously served to keep the tissue of this region in a stretched condition. Closing movement in Mimosa appears to be due largely to a contraction of the cells of the concave region, while closing movement in Dionaea is due largely to an expansion of the cells of the convex half. In Dionaea there is probably also an outward passage of water from the cells of the concave region, as in Mimosa, but in Dionaea this water appears to pass into the cells of the concave region instead of into intercellular spaces. Another case in point is the rapid contraction movement of the stamens of the Cynareae, due, according to Pfeffer (1906), to passage of water from cells to intercellular spaces, as in Mimosa.

Superficially, the mechanism of movement in Dionaea resembles more closely that exhibited by tendrils than it does the mechanism of the movement just mentioned. Fitting (1903) observed that curvature in tendrils is due to change in the rates of growth on the opposite sides of the organ. According to this writer there is here, as in Dionaea, a pronounced temporary acceleration of the growth of the convex half, while, also as in Dionaea, the average rate of growth is also increased. After a temporary stimulation the tendril straightens and at the same time ceases to elongate. The opening of Dionaea leaves is also due to an increased rate of growth on the concave side.

The geotropic and heliotropic curvatures of growing organs are also due to unequal rates of growth on the opposite sides (Pfeffer, 1906). In this case growth is increased on the convex side but, according to the measurements of Sachs, there is a retardation of the average rate. If, as seems likely, there is a decrease in osmotic pressure in the concave region of Dionaea leaves and an increase of pressure in the opposed region, this feature of the movement in Dionaea is similar to the geotropic movements of pulvini that have ceased growing. According to Pfeffer (1906) Hilliard found, by plasmolysis, that geotropic curvature in such organs is accompanied by a decrease in osmotic pressure on the concave side and an increase on the convex side. If, on the other hand, the increase in the dimensions of the convex side of the leaf of Dionaea is connected with an increased extensibility of the cell walls, this is similar to the stretching of the convex side of growing organs showing geotropic and heliotropic curvatures, where the stretching is soon fixed by growth.

It thus seems that the phenomena connected with the mechanism of movement in Dionaea leaves are, except for the rate at which movement occurs, very similar to those shown in geotropic and heliotropic curvatures, and that there is as great a similarity between these two types of movement as there is between those shown by Dionaea and by Mimosa. The movement in Dionaea may perhaps be considered as intermediate in character between such movements as are exhibited in geotropic and heliotropic curvatures, on the one hand, and the rapid movement resulting from mechanical stimulation in Mimosa on the other.

Duration of the Effect of Stimulation

Macfarlane (1902) states that at least two mechanical stimuli are necessary to produce closure of Dionaea leaves and that the number of stimuli required increases as the time period between successive stimuli is lengthened. Brown and Sharp (1910) found that at temperatures around 2° C. two stimuli are usually necessary for closure, but that at 35° C. a single stimulus is frequently sufficient. These writers state that if stimuli are applied at intervals of from 20 seconds to 3 minutes the number of these necessary to produce closure increases with the time elapsing between the application of the stimuli. They also showed that if one of the sensitive bristles on the inner surface of the leaf of Dionaea is stimulated by more than a very slight touch, the effect is independent of the amount of bending of the bristle, each such stimulus, regardless of its intensity, producing the maximum effect for a stimulus of that kind. It is therefore easy to apply several successive stimuli at equal time intervals, and to repeat such a series with different time intervals, the intensity of the force applied to the sensitive bristle requiring no serious attention in this case. The present section deals with the results of a study carried out in the way just suggested.

In each experiment a series of mechanical stimuli were applied to a single sensitive bristle, at equal intervals of time, and record was made of the number of stimuli required to produce the first visible response, and of the number required to produce complete closure of the leaf. In different experiments the length of the equal time intervals ranged from 20 seconds to 20 minutes. All these experiments were carried out with a temperature of about 21° C. On account of the general importance of this sort of data in the physiology of response, and because of the tediousness of such experiments, the results of these series are presented in full in Table VII. They are summarized by averages in Table VIII.

TABLE VII

Response of Dionaea Leaves to Repeated Contact Stimuli, With Various Lengths of Time Intervening Between the Stimuli

Time Period Between Stimuli	Experiment No.	No. of Stimuli Before Complete Closure	Time Between First Stimulus and Complete Closure	No. of Stimuli Before Beginning of Movement	Time Between First Stimulus and Beginning of Movement	No. of Stimuli Between Beginning of Movement and Complete Closure	Time Between Beginning of Movement and Complete Closure
min.			*min.*		*min.*		*min.*
0.33	1	2	0.33	2	0.33	....	....
	2	2	0.33	2	0.33	....	....
	3	2	0.33	2	0.33	....	....
	4	2	0.33	2	0.33	....	....
	5	2	0.33	2	0.33	....	....
	6	2	0.33	2	0.33	....	....
	7	2	0.33	2	0.33	....	....
	8	2	0.33	2	0.33	....	....
	9	2	0.33	2	0.33	....	....
	10	2	0.33	2	0.33	....	....
	Average	2	0.33	2	0.33	....	....
0.67	1	2	0.66	2	0.66	....	....
	2	2	0.66	2	0.66	....	....
	3	2	0.66	2	0.66	....	....
	4	2	0.66	2	0.66	....	....
	5	2	0.66	2	0.66	....	....
	6	2	0.66	2	0.66	....	....
	7	3	1.33	2	0.66	1	0.66
	8	3	1.33	2	0.66	1	0.66
	9	3	1.33	2	0.66	1	0.66
	10	3	1.33	2	0.66	1	0.66
	11	4	2.66	4	2.66	....	....
	12	5	3.33	3	2.00	2	1.33
	Average	2.75	1.27	2.25	0.94	0.50	0.33
1	1	3	2	2	1	1	1
	2	3	2	2	1	1	1
	3	3	2	2	1	1	1
	4	3	2	2	1	1	1
	5	4	3	3	2	1	1
	6	4	3	3	2	1	1
	7	4	3	3	2	1	1
	8	5	4	4	3	1	1
	9	5	4	4	3	1	1
	Average	3.77	2.77	2.77	1.77	1.00	1.00
2	1	5	6	4	6	1	2
	2	6	10	5	8	1	2
	3	6	10	5	8	1	2
	4	6	10	5	8	1	2
	5	6	10	5	8	1	2
	6	7	12	5	8	2	4
	7	7	12	6	10	1	2
	8	7	12	6	10	1	2
	9	7	12	6	10	1	2
	Average	6.33	10.66	5.22	8.44	1.11	2.22

TABLE VII—*Continued*

Time Period Between Stimuli	Experiment No.	No. of Stimuli Before Complete Closure	Time Between First Stimulus and Complete Closure	No. of Stimuli Before Beginning of Movement	Time Between First Stimulus and Beginning of Movement	No. of Stimuli Between Beginning of Movement and Complete Closure	Time Between Beginning of Movement and Complete Closure
min.			*min.*		*min.*		*min.*
3	1	5	12	4	9	1	3
	2	5	12	4	9	1	3
	3	8	21	4	9	4	12
	4	8	21	6	15	2	6
	5	9	24	7	18	2	6
	6	9	24	8	21	1	3
	Average	7.33	19.0	5.50	13.50	1.83	5.50
4	1	6	20	4	12	2	8
	2	8	28	7	24	1	4
	3	9	32	7	24	2	8
	4	9	32	7	24	2	8
	5	9	32	7	24	2	8
	6	9	32	7	24	2	8
	Average	8.33	29.33	6.50	22	1.83	7.33
5	1	7	30	5	20	2	10
	2	7	30	5	20	2	10
	3	7	30	5	20	2	10
	4	7	30	5	20	2	10
	5	8	35	6	25	2	10
	6	10	45	6	25	4	20
	7	12	55	6	25	6	30
	8	12	55	6	25	6	30
	Average	8.75	38.75	5.50	22.50	3.25	16.25
7	1	6	35	5	28	1	7
	2	9	56	7	42	2	14
	3	9	56	7	42	2	14
	4	11	70	8	49	3	21
	5	12	77	9	56	3	21
	6	16	105	10	63	6	42
	Average	10.50	66.50	7.66	46.66	2.83	19.83
10	1	6	50	5	40	1	10
	2	12	110	8	70	4	40
	3	12	110	9	80	3	30
	4	12	110	9	80	3	30
	5	13	120	10	90	3	30
	6	15	140	12	110	3	30
	7	15	140	12	110	3	30
	8	16	150	15	140	1	10
	Average	12.62	116.25	10.00	90.00	2.62	26.25

TABLE VII—*Continued*

Time Period Between Stimuli	Experiment No.	No. of Stimuli Before Complete Closure	Time Between First Stimulus and Complete Closure	No. of Stimuli Before Beginning of Movement	Time Between First Stimulus and Beginning of Movement	No. of Stimuli Between Beginning of Movement and Complete Closure	Time Between Beginning of Movement and Complete Closure
min.			*min.*		*min.*		*min.*
15	1	10	135	8	105	2	30
	2	11	150	8	105	3	45
	3	13	180	8	105	5	75
	4	15	210	10	135	5	75
	5	16	225	8	105	8	120
	6	16	225	8	105	8	120
	7	16	225	10	135	6	90
	8	17	240	11	150	6	90
	9	20	285	9	120	11	165
	10	22	315	14	195	8	120
	Average	15.60	219.00	9.40	126.00	6.20	93.00
20	1	14	260	12	220	2	40
	2	16	300	13	240	3	60
	3	16	300	13	240	3	60
	4	18	340	13	240	5	100
	5	19	360	13	240	6	120
	6	19	360	15	280	4	80
	7	20	380	14	260	6	120
	8	20	380	19	360	1	20
	9	23	440	17	220	6	120
	10	26	500	15	280	11	220
	Average	19.10	362.00	14.40	268.00	4.70	94.00

TABLE VIII

Response of Dionaea Leaves to Repeated Contact Stimuli, With Various Lengths of Time Intervening Between the Stimuli, Being a Summary of the Averages from Table VII

Time Period Between Stimuli	No. of Stimuli Before Complete Closure	Time Between First Stimulus and Complete Closure	No. of Stimuli Before Beginning of Movement	Time Between First Stimulus and Beginning of Movement	No. of Stimuli Between Beginning of Movement and Complete Closure	Time Between Beginning of Movement and Complete Closure
min.		*min.*		*min.*		*min.*
0.33	2.00	0.33	2.00	0.33	0.00	0.00
0.66	2.75	1.27	2.25	0.94	0.50	0.33
1	3.77	2.77	2.77	1.71	1.00	1.00
2	6.33	10.66	5.22	8.44	1.11	2.22
3	7.33	19.00	5.50	13.50	1.83	5.50
4	8.33	29.33	6.50	22.00	1.82	7.33
5	8.75	38.75	5.50	22.50	3.25	16.25
7	10.50	66.50	7.66	46.66	2.83	19.83
10	12.62	111.25	10.00	90.00	2.62	26.25
15	15.60	219.00	9.40	126.00	6.20	93.00
20	19.10	362.00	14.40	268.00	4.70	94.00

From a study of these tables it is seen that if two stimuli are applied with an interval of 20 seconds (0.33 min.) between them, closure occurs immediately after the latter stimulus, taking only a few seconds. When the interval between the two stimuli is forty seconds (0.67 min.) there may be only a partial closure following the latter stimulus. As the interval between the stimuli becomes greater the number of stimuli required for closure increases, as do also the number of stimuli necessary before a visible effect is produced, and the number between the first partial closure and complete closure. When the intervals are very long closure is so gradual as to be quite imperceptible; nevertheless the lobes slowly approach each other and complete closure is finally attained, even with intervals of 20 minutes, which were the longest intervals employed. In one case 26 stimuli were applied, the total period of time between the occurrence of the first stimulus and complete closure being 8 hours and 20 minutes. In this experiment three hours and 40 minutes elapsed between the occurrence of the first visible change and the attainment of complete closure. Between this slow movement and the ordinary rapid closure there is a gradual intergradation as is shown by the data of Tables VII and VIII.

Here also there is an apparent similarity between the phenomena of leaf closure in Dionaea and those of geotropic curvature; in the latter case the reaction time may also be lengthened or shortened. Thus Czapek (1895) has shown that by subjecting the roots of *Vicia faba* to varying amounts of centrifugal force the reaction may take place in from three fourths of an hour to 8 hours. Fitting (1905) has investigated the summation of geotropic stimuli of short duration and has found that single short stimuli are not sufficient to produce curvature but that curvature takes place even though the lengths of the individual stimuli are greatly shortened, provided only that the length of time between the stimuli is not too great. Brown and Sharp (1910) have shown that in the case of Dionaea there may be a summation of individual mechanical or electrical stimuli of low intensities. In one case, when feeble electrical stimuli were applied at intervals of 15 seconds, movement did not occur until after the twenty-eighth stimulus.

The relation between the number of stimuli necessary for the closure of Dionaea leaves and the length of the time interval between the consecutive stimuli is shown graphically in figure 1, in which the ordinates represent the time intervals between the successive stimuli and the abscissas denote the average number of stimuli required to produce closure.

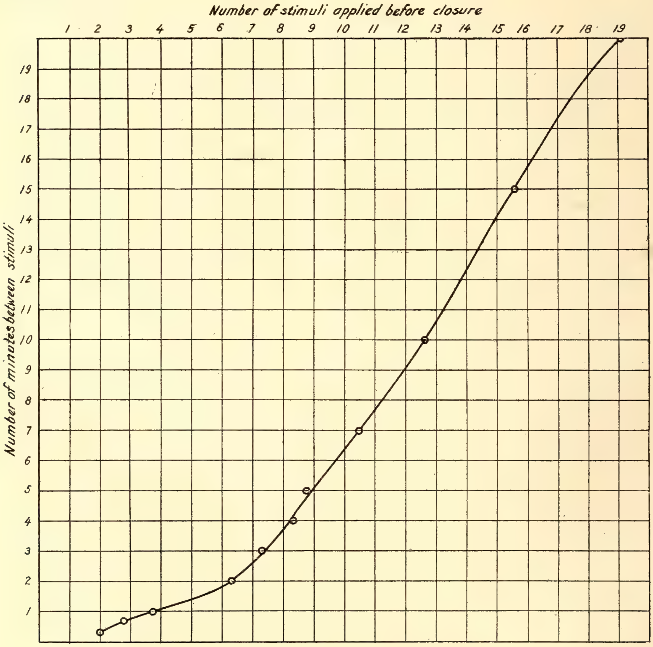

FIG. 1. Graph showing relation between the number of successive stimuli necessary for the closure of the leaf of Dionaea, and the time interval separating the individual stimuli; data from Table VIII.

This graph shows at once that the number of stimuli required for complete closure of the leaf is not proportional to the length of time intervening between the successive stimuli, excepting with time intervals of from 2 to 4 minutes; this portion of the curve, and this portion only, has a slope of approximately 45°. With time intervals of from 20 seconds to 2 minutes the curve is less steep (or more nearly horizontal) and with intervals longer than 3 minutes it is somewhat steeper (more nearly vertical). The curve is thus roughly divided into two portions, the upper of which is steeper than the lower. This main bend in the curve may be of considerable importance, but the physiological conditions determining it appear to be as yet quite unknown.

SUMMARY

The closure of Dionaea leaves is due largely to an increase in the size of the cells of the ventral or convex region, this increase being due to stretching of the cell walls, which soon becomes fixed by growth.

The opening of the leaf is due to slow enlargement, by growth, of the cells of the dorsal or concave region.

Stimulation of the leaf results in a greatly accelerated rate of growth.

Stimulation appears to be immediately followed by a decrease in the osmotic pressure of the cells of the dorsal region, resulting in a passage of water from these cells to those of the ventral region.

A large quantity of starch is deposited in the cells of the dorsal region soon after closure occurs.

Leaves that have been killed in boiling water just after closure, open if transferred through alcohol to xylene and close again when replaced in water.

The mechanism of movement in Dionaea leaves shows many points of apparent similarity to that of geotropic curvatures.

At 21° C. two mechanical stimuli are usually necessary to produce closure in these leaves, but if the time interval between the successive stimuli is increased the number of stimuli necessary for closure also increases, though the latter increase is not proportional to the total time period involved in the reaction.

In one case when stimuli were applied at 20-minute intervals, closure was not complete until eight hours and twenty minutes after the application of the first stimulus.

BUREAU OF SCIENCE,
MANILA, P. I.

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THE SPECIFICITY OF PROTEINS AND CARBOHYDRATES IN RELATION TO GENERA, SPECIES AND VARIETIES*

Edward Tyson Reichert

It has long been known that both animals and plants can be definitely grouped upon the basis of the presence or absence of some particular kind of substance or group of allied substances, etc.: animals, in accordance with the presence or absence of haemoglobin, haemocyanin or myogen, etc.; and plants as to whether or not they contain starch, glycogen, tannic acid, some peculiar forms of toxic substances or some particular form of protein, etc. As regards proteins, it has been found that proteins of seeds from different plant sources are not identical and that similar or apparently identical proteins are found only in seeds that are botanically closely related. From the results of a series of elaborate investigations that are being carried on under the auspices of the Carnegie Institution of Washington we may go farther than this gross differentiation of groups and state that a given substance such as haemoglobin or starch may exist in modified forms which in number may infinitely exceed the number of known genera, species and varieties, and that from present indications these modifications are specifically taxonomic.

In order to have clear conceptions of the possibilities of such inconceivably numerous forms of a single substance it is essential that we recall to mind certain salient facts regarding modern conceptions of molecular structure. It will be remembered that when substances have the same kinds and the same number of each kind of atoms they are isomers, and have the same molecular formula; that if isomers so differ in their properties as to indicate that they are different

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* Invitation paper read before the Botanical Society of America and affiliated societies at Columbus, December 29, 1915.

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substances, the differences are owing to variations in the linkages of the components, which differences are expressed by structural formulae that set forth the linkages in the two dimensions of space; and that when substances have the same molecular and structural formulae but differ in their properties, the differences are due to variations in the arrangements of the components in three dimensions of space, that is, in the configurations of the molecules, which differences are expressed by space formulae. Bodies belonging to the last group are known as stereoisomers or corresponding substances, that is, each kind of substance may exist in a number of forms, all of which forms have the same molecular formula, the same structural formula, and the same fundamental properties in common, but each in accordance with variations in intramolecular configuration has certain individualities which distinguish it from the others.

There are many known substances that exist in stereoisomeric forms, and it has been found that the number of possible forms of each substance is dependent upon the possible number of variations of the arrangements of the molecular components in the three dimensions of space, or, in other words, of variations of molecular configuration, the possible number in case of each substance being capable of mathematical determination. Thus, we find that serum albumin may exist in as many as a thousand million forms. Haemoglobin, the red coloring matter of vertebrate blood, is a far more complex carbon compound than serum albumin, and theoretically may exist in forms whose number is beyond human conception, running into millions of millions. The same is true of starch.

Elsewhere[1] have been set forth with sufficient fullness the hypotheses and theories that underlie an elaborate series of researches which have as their primary object an investigation of corresponding substances obtained from various forms of plant and animal life in relation to taxonomy, sports, mutations, reversions, heredity in general, tumor formation, etc., and nothing more seems necessary in the present address than to state that these researches have as their essential basis the conception that in different organisms the corresponding complex organic substances which constitute the supreme structural components of protoplasm and the major synthetic products of protoplasmic activity are so different as to impart specific peculiarities to the organisms in which they are formed, to be as distinctive of the genus or species or sex as the data of the systemist, and to be determinate of the specificity of the protoplasm itself.

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[1] Publications 116 and 173 and the Year-Books 9 to 13 of the Carnegie Institution of Washington.

It follows from what has been stated that haemoglobin may not only exist in nature in countless forms, but also that each form may be absolutely characteristic of the genus and species.

In an investigation of the haemoglobins it was found that these substances exhibit differences in solubility, decomposibility in relation to putrefactive organisms, quantity of water of crystallization, decomposibility in relation to various chemical reagents, extinction coefficients and quotients, crystallizability, and form and habit of crystallization. The characters of the crystals were especially studied, particularly the forms and habits of crystallization, the peculiarities of twinning, and the "optical reactions," which latter as determined by the aid of the polarizing microscope may be found analytically to be as definite and exact as the reactions obtained by the conventional methods of the chemist. It was found, for instance, in these studies which embraced examinations of specimens of haemoglobins from over 100 species representing many genera and families:

1. That there is a common structure of the haemoglobin molecule whatsoever the source of the haemoglobin.

2. That the crystals of the species of any genus belong to a crystallographic group which represents a generic type.

3. That the crystals of each species of a genus when favorably developed can be distinguished from those of other species of the genus.

4. That the crystals of different generic groups differ as definitely and specifically as those of crystalline groups of mineral substances differ chemically, and as generic groups differ zoologically or botanically.

5. That by means of the peculiarities of haemoglobins phylogenetic relationships can be traced, as has been found in the case of the bear and certain other animals.

Subsequent studies with other substances, especially with animal and plant proteins, a large number of starches, some glycogens and chlorophyls and other complex metabolites, have elicited confirmatory results and even extended the data of the haemoglobin research.

The investigations with the starches were necessarily carried on by methods that are quite different from those employed in the study of the haemoglobins. Although the starch granule is a spherocrystal that lends itself to crystallographic study, very little can be learned of its molecular characters that is of usefulness in the differentiation of various starches. Other methods, however, offer very satisfactory means of study, especially those which elicit molecular differences by means of peculiarities of gelatinization. These methods, all microscopic, have included inquiries into histological characters, polariscopical, iodine and aniline reactions; temperatures of gelatinization; and quantitative and qualitative gelatinization reactions with a variety of chemical reagents which represent a wide range of difference in molecular composition.

Each starch property, whether it be manifested in peculiarities in size, form, hilum, lamellation or fissuration, or in reactions to light, or in color reactions with iodine or anilines, or in gelatinization reactions with heat or chemical reagents, is an expression of an independent physico-chemical unit-character that is an index of specific peculiarities of intramolecular configuration, the sum of which is in turn an index which expresses specific peculiarities of the constitution of the protoplasm that synthetized the starch molecule. The unit-character represented by the form of the starch grain is independent of that of size; that of lamellation independent of that of fissuration, etc. This is evident in the fact that in different starches variations in one may not be associated with variations in another, and that when variations in different properties are coincidently observed they may be of like or unlike character. Gelatinizability is one of the most conspicuous properties of starch and it represents a primary physico-chemical unit-character, which character may be studied in as many quantitative and qualitative phases as there are kinds of starches and kinds of gelatinizing reagents, the phenomena of gelatinization by heat being distinguishable from those by a given chemical reagent, and those by one reagent from those by another, and those of one starch by a given reagent from those of another starch. The gelatinization of the starch grain is certainly not, as is commonly supposed, a manifestation of a simple process of imbibition of water, such as occurs in the swelling of particles of dry gelatin or albumin, but in fact a very definite chemical process corresponding to that which occurs in the swelling of liquid crystals, and which must vary in character in accordance with the reagent entering into the reaction. It therefore follows, as a corollary, that the property of gelatinizability of any specimen of starch may be expressed in as many independent physico-chemical unit-character-phases as there are reagents to elicit them.

By these methods physico-chemical unit-characters and unit-character-phases can be reduced to figures from which charts can be constructed which show in the case of each starch that the sum-total of these values is as distinctive of the kind of starch and plant source as are botanical characters of the plant. In determining these values certain precautions must sedulously be observed in order to obtain dependable results. Thus, in the polarization, iodine and aniline reactions, definite though arbitrary standards of comparison must be adopted. This can crudely but satisfactorily be accomplished by selecting as standards three or four starches which exhibit desired gradations of value, and constructing a scale by the aid of which values can be reduced in figures. In all of the gelatinization reactions the examinations must be made on the stage of the polarizing microscope. In determining the temperatures of gelatinization especial care must be exercised in regard to uniformity in the rapidity with which the preparations are heated, together with such other precautions as have been found essential in determining "melting points." In the reactions with the chemical reagents it is absolutely essential that immediately upon the addition of the reagent to the starch on the slide the preparation be kept air-tight to avoid changes in concentration of the reagent by loss or addition of water, and to avoid effects of oxidation. It goes without saying that in the iodine, aniline and chemical-reagent experiments it is necessary to use definite and constant proportions of reagent and starch. Finally, inasmuch as the starch of any given plant varies somewhat in relation to season, rest and activity, the part of the plant in which it is formed, etc., it obviously is important in comparative experiments such as those under consideration to obtain specimens from corresponding parts of plants and under other corresponding conditions. In the present researches all of the starches were prepared from bulbs, tubers, rhizomes, etc., in the resting state. Each preparation was obtained from a number of specimens, usually 25 to 50, so that each is representative of the plant source.

The measure of value to be attached to the results of researches that are carried out along such exceptional lines, and by means of such seemingly gross methods of study, must naturally rest inherently upon the uniformity of the results of repeated experiments and upon the conformity of the results with established data of the systematist. As to the former, it need only be stated that when experiments have been repeated, even though under varying laboratory conditions as regards temperature and humidity, the results have been either identical or have differed so little as to be absolutely unimportant. As to the latter, it will be found that the records are to an astonishing degree in harmony with established botanical peculiarities, and that where perchance there may be departure the causes therefor are usually not far to seek. It perhaps is needless to state that as great care and technical skill may be required to conduct successfully such experiments as are demanded where the methods are in the conventional sense exact, and consequently that much training may be necessary before one is fitted to make permanent records.

In these researches it has been found desirable to record the quantitative gelatinization values in three kinds of charts, each presenting in its own particular and impressive way certain striking peculiarities which are not at all or not so well exhibited by another. One kind shows the progress of gelatinization in time-percent reaction curves of the starch with a given reagent; another, the differences in gelatinizability of different starches with a given reagent; and another, composite curves of reaction-intensities of a given starch with a number of agents and reagents, by means of which types of curves of varieties, species and genera are obtained.

From these investigations the following fundamental statements are deduced:

1. The results of the haemoglobin and starch researches are mutually confirmatory in proving the existence of different stereoisomeric forms that are specifically modified in relation to varieties, species and genera, in other words, stereoisomeric specificity in relation to taxonomy; and that such specificities indicate corresponding specificities of the protoplasms that give rise to these different forms.

2. The reaction-intensities of different starches with a given reagent vary within wide limits, and vary with each reagent independently of the variations of other starches.

3. The reaction-intensities of varieties of a species very closely correspond with those of the species and they are in accord with botanical characters.

4. The reactions of different species of a genus exhibit characters in conformity in general with the values of the distinguishing botanical characteristics of the members of the genus, the species-characters varying in degree of closeness or separation in accord with corresponding botanical peculiarities.

5. The reactions of the members of a genus constitute a well-defined group, the mean of the character-values constituting a distinct generic type.

6. When a genus consists of subgenera, or groups of rhizomatous and tuberous plants, or tender and hardy plants, etc., there may be as many subgeneric types as there are groups.

7. When subgeneric types exist they may be bridged in part by intermediate characters of a hybrid that is the offspring of members of such types.

8. The generic types belonging to a given family tend in general to exhibit closeness or separation in accord with established botanical data.

These researches have been of a purely exploratory character. No attempt has been made to establish by sufficiently repeated experiments and otherwise what may be accepted as character-constants, but rather to establish certain principles and a foundation or starting point for final investigation. In fact, there remains much to be done in the way of preliminary work before the final studies are begun. Particularly important is it to extend the period of observation, modify the concentrations of some of the reagents, introduce additional reagents, improve the polarization and the iodine and aniline methods or eliminate them, and study the stereoisomers of successive generations in relation to various conditions that influence heredity. Considerable advances must be made in order to demonstrate satisfactorily certain taxonomic differences that undoubtedly exist, and also to set forth satisfactorily these differences in the form of composite charts. In such composite charts as constructed up to the present no difference may be shown between two starches in a given reaction, yet the records may show that during the progress of gelatinization more or less marked differences were recorded. Hence, in taxonomic studies it is essential to consider collectively all three kinds of charts, and in association with the histological characters and qualitative reactions. In other words, in the ultimate analyses and comparisons we must utilize the sum-total of characters and character-phases.

In conclusion, it need scarcely be stated that limitations of time have made the presentation of so extensive a topic of a somewhat scrappy character, and that notwithstanding the incompleteness and various defects of these researches facts and principles of epochal importance in relation to the mechanisms of living matter have been brought to light, and hence the way shown for developments of the greatest moment in normal and abnormal biology, as for instance:

1. It seems obvious that we have found a strictly scientific basis for the classification of plants and animals.

2. There are manifestly certain striking applications that are of the greatest fundamental importance in the study of phylogeny, mutations, reversions, sex, malformations, phenomena of heredity in general, etc. (For an application, see article on *The Germplasm as a Stereochemic System*, *Science*, **40**: 649–661. 1914.)

3. The discovery of the existence of highly specialized stereoisomers has brought before us one of the most remarkable and unsuspected phenomena of living matter, and one which leads us directly to the constitutions of various forms of protoplasm and the peculiarities of vital phenomena that are dependent upon these differences.

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MECHANICS OF DORMANCY IN SEEDS*

Wm. Crocker

Introduction

Dormancy in plants is common in three organs, seeds, spores, and buds. In this paper I shall limit myself entirely to the discussion of dormancy in seeds for three reasons: far more critical and analytical work has been done on dormancy in seeds than upon the other two organs; obscure correlation effects do not play as prominent a part as in buds; and I am best acquainted with this phase of the subject.

The title of this paper might imply that I am aligning myself with the mechanists as against the vitalists; such is not the case. The vitalists can justly claim that in growth we have as yet explained so little by known chemical and physical laws and have so much still to explain on this basis or on the basis of newly discovered laws that the title "Mechanism of Growth" would imply a very meager discussion of our knowledge of this process. To date we have to discuss growth mainly in physiological rather than mechanistic terms. The situation is rather different with the dormancy of seeds. Here so far as the work is analytical, it indicates that dormancy is brought about by factors inhibitory to general processes preceding or accompanying growth.

In plants of the temperate zone, the seeds generally have a rest period. Howard[1] finds that more than 75 percent of the species, wild and cultivated, growing around Columbia, Missouri, have a distinct period of dormancy. The rest period is more general and much more persistent among wild than cultivated forms. Selection resulting from methods of cultivation have largely disposed of this character in domesticated forms, as is well illustrated by the two closely related species, *Avena fatua* and *A. sativa*.[2] The former is delayed in its germination until the spring following ripening, constituting it one of the bad weeds of Minnesota, the Dakotas and western Canada; while the latter grows rather readily in the late summer and fall and is killed by the severe freezing of winter. When in the eighties the Germans[3] began introducing the wild legumes as forage crops they found one of the great drawbacks was the persistent dormancy of the seeds. Seeds of many plants have a dormancy that persists only until the spring following ripening. Others are carried over two or more winters in the quiescent condition, while still others have the germination of a single crop distributed over the growing seasons of from one to many years.

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* Invitation paper read before the Botanical Society of America and affiliated societies at Columbus, December 29, 1915.

[1] Howard, Mo. Agr. Exp. Sta. Res. Bull. 17.

[2] Criddle, Dept. Agr. Ottawa, Bull. S-7.

The maximum time that seeds can lie in the ground in a dormant but viable condition is a matter of much interest and one that has received considerable attention. Numerous observations[4] of the vegetation appearing upon soil freshly turned up by the digging of wells, ditches, the removal of buildings and the plowing of old meadows and pastures, indicates that various seeds may lie in the soil quiescent and viable for 25 or even 50 years. Peter took samples of soils at various depths from forests that had been grown upon meadow and farm lands for known periods ranging from 20 to 150 years and subjected these soil samples to germinative conditions. The samples from younger forests showed seeds of farm weeds and meadow plants prevalent, while in the older forests there was a gradual displacement by seeds of forest vegetation, and in the oldest forests only the latter appeared. The soil from forests 20 to 46 years old produced 41 species of farm weeds and 35 of meadow plants. In forests over 100 years old he still found *Hypericum humifusum*, *Stellaria media* and *Juncus bufonius*. From his work Peter concludes that seeds of certain plants may lie quiescent in the soil for more than 50 years still capable of germination when supplied proper temperature, light and moisture conditions. Perhaps Ewart has been over severe in his criticism of this rather careful work notwithstanding the possible errors necessarily involved in the method. However this may be, later and conclusive experiments upon buried seeds tend to confirm Peter's conclusions.

Beal[5] has found that out of 22 species representing 16 families, including common plants, mostly weeds, 11 species did not retain their viability after 5 years in the soil, but some seeds of 8 species were still viable after 30 years. It is interesting to note these: *Amaranthus retroflexus, Brassica nigra, Bursa Bursa-pastoris, Lipidum virginicum, Oenothera biennis, Rumex crispus, Setaria glauca, Stellaria media.* The appearance of *Stellaria media* in this list is confirmatory of Peter's conclusions.

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[3] Michalowski, Würt. Wochenbl. Landwirtsch. **13**: 175. 1894.

[4] Peter. Nachr. Königl. Ges. Wiss. 673. 1893; 373. 1894.

[5] Beal. Vitality of Seeds Buried in the Soil. Amherst, Mass.

The work of Duvel on the viability of buried seeds, because of the number and range of species represented, the planned duration of the project and the approach to natural conditions, is to be a notable work in this line. He has published[6] the record of earlier tests and he and his co-worker, Mr. Goss, have kindly allowed me to examine the results of the ten-year period. Out of 105 species, representing 25 families, more than half the species, representing more than two-thirds of the families, still show viable seeds ranging from 1 percent in some to nearly 100 percent in others. Of the eight species Beal finds retaining their viability 30 years, the six which are represented in Duvel's list are still viable after ten years; two of them giving about 90 percent germination. Excepting hard forms, seeds of cultivated plants in general show but transient longevity in the soil in contrast to the rather persistent longevity of wild seeds. In general the records on buried seeds confirm the conclusions based on observations and on work of the type of Peter's. In this connection it is interesting to find the claims of the farmers of the South Downs,[7] that *Brassica nigra* seed can lie in the soil for years still capable of growth when supplied proper conditions, confirmed by the exact work of Beal and Duvel.

Theories of Longevity

The question of the nature of the process involved in the gradual loss of viability of seeds with the elapse of time and the effect of conditions upon the rate of this process is of first importance in a discussion of dormancy, for they set the maximum duration of this state. It is well to consider first, seeds that will withstand drying and to consider the loss of viability in approximately air-dry condition. Two explanations capable of experimental investigation have been offered: exhaustion of stored foods and degeneration of the digestive and oxidizing enzymes.[8] Both of these explanations have proved incorrect, for foods and enzymes are present in almost full force for some years after viability is lost.

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[6] Duvel. U. S. Dept. Agr., Bur. Pl. Ind. Bull. 83. 1905.

[7] Kidd. Proc. Roy. Soc. **87**B: 408 and 625. 1914.

[8] Crocker and Groves. Proc. Nat. Acad. Sci. **1**: 152. 1915. Also unpublished work by the latter.

Ewart[9] says "Longevity . . . depends upon how long the inert proteid molecules into which the living protoplast disintegrates when drying, retain the molecular grouping which permits of their recombination to form the active protoplasmic molecules when the seed is moistened and supplied with oxygen." In this form of statement Ewart's theory is quite beyond experimental investigation. Groves[8] and I have suggested that loss of viability in seeds approaching the air-dry condition is due to the slow denaturing, or coagulation, of certain protoplasmic proteins of the embryo—a statement resembling Ewart's in some ways but one capable of experimental study. The extreme lability of protoplasmic proteins as shown by Lepeschkin[10] would suggest their being denatured far sooner than such proteins as make up or at least are associated in the main with the rather stable oxidizing and digestive enzymes.

The evidence for this hypothesis was gained by studying the relation between temperature and life duration of wheat seeds at temperatures ranging from 50° to 100° C., since this range of temperature gives life durations convenient for experimental purposes.

The following results of this work show the likeness between life duration of wheat grains (*Triticum sativum*) at temperatures above 50° C. and the coagulation time of proteins. At given temperatures the life duration fell with increased water content. When the logarithms of the life duration in minutes was plotted on the abscissae and the temperatures in degrees centigrade on the ordinates a straight-line curve resulted showing that life duration is a logarithmic function of the temperature. The formula applied by Lepeschkin as a time-temperature formula for the coagulation of proteins as well as a temperature-life duration formula for imbibed cells applies also as a temperature-life duration formula for wheat grains ($T = a - b \log Z$, in which T = degrees centigrade, a and b are constants and Z is time in minutes). For rise in temperature the rate of loss of longevity rises much faster than assumed by the van't Hoff law—a law applying rather generally to the rate of chemical reactions *in vitro* as well as to many processes in the living organism. While this law assumes increased speed of two to three fold for a rise of 10° C., wheat containing

[9] Ewart. On the Longevity of Seeds. 210 pp. Victoria, Australia, 1908.

[10] Lepeschkin. Ber. Deutsch. Ges. Bot. **30**: 703. 1913.

from 9 to 12 percent moisture showed a coefficient of 7 to 8, and that containing 18 percent moisture 10 to 16 for 10° C. rise in temperature. For life duration of barley seeds, containing considerable moisture, Goodspeed got a coefficient of 10 to 16 and Loeb and Moore on various sea forms found coefficients ranging from 500 to 1,450. It is interesting to compare the above temperature coefficients for life duration with those for the coagulation of various proteins. The latter vary from 6 to 635 according to the range of temperature, moisture content and protein used.

The evidence is good then that at temperatures above 50° C., with air-dry seeds or those containing somewhat less or considerably more water, the loss of viability is a matter of the denaturing of embryo proteins.

What about loss of viability at laboratory temperatures and temperatures prevalent in natural conditions? When we compare the longevity of wheat, containing 12 percent moisture, as calculated from our measurements at high temperatures either on the basis of the Lepeschkin formula or of the temperature coefficient found, the values are of about the same magnitude as those observed for wheat by White.[11] There is still need of much work on several long-lived and short-lived seeds of which we have fairly reliable records of longevity, to see whether the calculated longevities from measurements at high temperatures by the two methods mentioned above, tally well with observed longevities.

To make concrete the indicated significance of temperature in longevity of seeds let us assume that wheat with a fixed water content at 20° C. has a longevity of 16 years and that the temperature coefficient of 8 still applies at low temperatures. On these assumptions the longevity at 30° C. would be two years, at 10° C. 128 years and at 0° C. 1,024 years.

The total of our results gives considerable grounds for believing that at low and *equable* temperatures and moisture contents medium-lived grains like wheat may retain their vitality several centuries.[12] I am confident, however, that this coagulation theory of longevity has much greater value in opening a line of attack than in matters already established.

The statements above apply to seeds approaching the air-dry condition, but as we shall see later not all seeds lying dormant in the soil for thirty years or more are in this condition. Lepeschkin finds evidence for the presence of a re-dispersal process in fully imbibed plant cells. At ordinary temperatures he believes this increases many fold the life duration of plant cells. It and many other factors such as change in reaction of the cell may modify the speed of coagulation. Here too it is possible that carbon dioxide or other narcotics produce a state of rigor thereby greatly lengthening the life of the seed as is indicated by the work of Kidd. It is possible that seeds like *Amaranthus retroflexus* and *Brassica nigra*, lying in the soil for thirty years or more nearly or quite saturated with water, entirely consume their stored food in respiration and die of starvation. It is not known whether these seeds remain alive longer in this condition or in air-dry storage.

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[11] White. Proc. Roy. Soc. London 81B: 417. 1909.

[12] It is probable that the action of oxygen as well as noxious gases must be avoided. Becquerel. Ann. Sci. Nat. Bot. IX 5: 193. 1907.

The changes involved in the rapid loss of vitality by seeds that will not withstand drying are still more obscure. The nature of the injury produced by drying is also entirely unknown.

Causes of Dormancy

In securing delayed germination of seeds, plants are not limited to the dead monotony of one method. As one studies the problem more fully he wonders whether there is any conceivable method of securing delay not made use of in one plant or another. I believe that failure to grasp the variety of methods and the counter attempts to explain all delays by one or at most two methods, is the main source of the controversy, error and confusion that have prevailed in this field.

We will now consider two general topics: (1) Methods of securing dormancy and (2) methods of overcoming it, or the action of forcing agents upon dormant seeds. The two topics are far from distinct. Certain subheads under either topic could be shifted to the other without violence, thus constituting an extremely complex interrelation between the general topics and the subheads under them. The classification here offered is for the convenience of discussion and must be subject to change with growth of knowledge in the field. Nevertheless, I believe some such classification very desirable at this time in clearing up a chaotic situation and in giving future experimentation direction and aim.

Methods of Securing Dormancy

As to the mechanism by which delay in germination of mature seeds is secured when they are placed under ordinary germinative conditions, we will consider the following: (1) Rudimentary embryos that must mature before germination can begin; (2) complete inhibition of water absorption; (3) mechanical resistance to the expansion of the embryo and seed contents by enclosing structures; (4) encasing structures interfering with oxygen absorption by the embryo and perhaps carbon dioxide elimination from it, resulting in the limitation of the processes dependent upon these; (5) a state of dormancy in the embryo itself or some organ of it, in consequence of which it is unable to grow when naked and supplied with all ordinary germinative conditions; (6) combinations of two or more of these; (7) assumption of secondary dormancy.

Primary Dormancy.—(1) It has been shown that at ripening many seeds have immature embryos[13] that must complete their development before germination can begin. Such embryos vary from an undifferentiated group of cells or perhaps in some cases a fertilized egg to mature embryos. A considerable range of immaturity often appears in a single species. This seed character appears more or less distributed through all the large groups of seed plants as is shown by a few illustrations: Gymnosperms—*Ginkgo biloba, Gnetum Gnemon;* Dicots—*Eranthis hiemalis, Ranunculus Ficaria, Corydalis cava, Stylidium, Gagea arvensis;* Monocots—*Hymenocallis speciosa, Paris quadrifolia, Erythronium denscanis.* The maturing of the embryo in some of the seeds of this class requires weeks and even months in the germinator, and delays germination to that extent. The immature embryos of this group must not be confused with those of certain saprophytes and parasites that germinate in the immature condition.

2. So-called hard seeds have coats that entirely prevent absorption of water. This phenomenon predominates in the Leguminosae but it is also common in Cistaceae and Malvaceae[9, 14] and has been observed in perhaps a dozen other families. In some species all the seeds of the crop are hard but more frequently only a portion. In either case in nature the germination of a single crop is distributed over a considerable period, probably in some cases amounting to many years. For instance, Nobbe found that red clover seeds placed in water do not

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[13] Goebel. Organographie der Pflanzen, 454. Jena, 1898–1901.

[14] Guppy. Studies in Seeds and Fruits, 585. London, 1912.

all swell at once, but a few at a time, a considerable percent remaining hard and viable even after a decade.[15] Hard seeds become permeable very slowly under dry storage, more rapidly in germinators under laboratory conditions, and apparently still more rapidly under widely fluctuating natural conditions. The means by which this resistance to water absorption is secured has been mainly studied in the legumes. Here a controversy has arisen as to whether it is due to the cuticle or to the " light zone " of modified cellulose of the palisade layer. Perhaps there is more evidence in favor of the former for small and medium sized seeds, and for the light zone or deeper layers of the palisade cells in larger seeds. Either will explain the rather general efficiency of surface abrasion or carbonization as means of forcing.[9, 14] Verschaffelt[16] has lately given this controversy an interesting turn. He finds that seeds of legumes in general have open micropyles that give free access to the water absorbing tissues below and that in addition in the subfamilies Caesalpinioideae and Mimosoideae rifts or lacunae communicating with water absorbing tissues below are present over much of the seed surface. Water fails to enter because it will not wet the walls of these openings. It will enter, however, if the seed is first soaked for an hour in ethyl alcohol and then placed in water. The alcohol readily enters the openings and furnishes a path for the inward diffusion of water. The efficiency of alcohol as a forcing agent varies much with different legumes, showing slight or no effect in many. It is likely Verschaffelt's explanation applies to a portion only of the legumes and perhaps not at all to other hard seeds. Moreover Ewart finds that in some hard seeds all layers of the integument are highly impervious to water and Guppy claims this is prevalent among hard-coated forms with large seeds. There is need here of much more investigation, especially of non-leguminous seeds.

For cultivated legumes Hiltner[17] claims that crops ripening in dry climates or in dry periods show a larger percent of hard seeds. On the other hand Harrington[18] finds that alfalfas and clovers, generally, grown on a variety of soils and ripening under most diverse climatic conditions, have about 90 percent of hard seeds if hulled by hand, but generally less than 20 percent if hulled by machine. The huller thus acts as a rather effective abrasive agent and the study of the machine hulled seeds concerning hardness throws more light upon the effectiveness of the machine than upon the seeds themselves.

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[15] Nobbe. See 17.

[16] Verschaffelt. Rec. Trav. Bot., Neerland. 9: 401. 1912.

[17] Hiltner. Land. Forst. Wirtsch. Kais. Gesundh. 3: 1. 1902.

[18] Harrington. U. S. Dept. Agr. Farmers' Bull. 676.

Concerning the biological significance of hard coats it may be stated that they place a family of seeds, otherwise extremely susceptible to injurious agents among those of the greatest longevity. Applying the principles mentioned in our coagulation conception of life duration, such seeds in the soil have ideal conditions for longevity —low equable temperature and moisture content. Moreover the impervious coats protect them against other organisms and the slow action of oxygen.

3. In the light of Müller's[19] recent work it is not strange that some seeds are held in a dormant state because the force of the expanding contents is not sufficient to rupture the coats. He found that in various seeds that germinate readily the outward pressure of the contents at the time of rupture was but slightly greater than the breaking strength of the water-saturated coat. Both lay in the region of 3 to 4 atmospheres. It has been shown that the breaking strength of filter paper, leather, Laminaria thallus and most other organic materials of colloidal nature rises or falls with a fall or rise in water content. Müller found the same true of seed coats. In the cases studied the breaking strength of the dry coats was several times the expanding force of the enlarging seed contents.

Of seeds inhibited in their germination by this method, *Alisma plantago*[20] and *Amaranthus retroflexus*[21] have been most fully studied. Water impervious coats play no rôle here for both absorb water very rapidly and reach saturation after about 5 hours' soaking. In the saturated condition it is estimated that the embryo of *Alisma plantago* due to imbibitional or osmotic absorption of water is exerting an outward pressure on the coats of about 100 atmospheres. In *Amaranthus retroflexus* the outward pressure is probably much less, but in both seeds the gel-like coats are considerably stretched, and the embryo rapidly extends beyond the bounds of the coat as soon as the latter breaks. Any time after maturity naked embryos of both these seeds are capable of immediate growth, showing no dormant period. The embryo of Alisma is a rather slow grower but that of Amaranthus very rapid. The latter shows a growth elongation of several hundred percent after 12 hours in the germinator under optimum conditions.

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[19] Müller. Jahrb. Wiss. Bot. 54: 529. 1914.

[20] Crocker and Davis. Bot. Gaz. 58: 285. 1914.

[21] ——— ———. Unpublished work.

The intact seeds of both these species are dormant when harvested. *Alisma plantago* remains so for years unless acted upon by some of the agents mentioned below, but Amaranthus in great part loses its dormancy after 2 or 3 months of dry storage. This "so-called" after-ripening in Amaranthus seems to involve hysteretic changes in the colloids of the coats by which they fall in elasticity or breaking strength. This hysteretic effect will be discussed under secondary dormancy.

Certain temperature relations of after-ripening in Amaranthus are of great interest. Temperatures above 40° C. will produce some germination in the ripe seeds harvested from green plants, and the minimum temperature falls as after-ripening progresses. Even fully after-ripened seeds have their minimum temperature lowered by removal of coat restrictions. As is true of gels, generally, the viscosity of gels of the coats lowers with rise of temperature and with it the breaking strength falls. The combined action of the hysteretical changes of the coat with this temperature effect on their breaking strength seems to explain the minimum temperature changes accompanying after-ripening.

Any treatments that greatly weaken the coats without injury to the embryos are good forcing agents for the dormant seeds of both Alisma and Amaranthus. Abrasion by various means, carbonizing with sulfuric acid or treatment with a great range in concentration of acids under proper adjustment of time are effective in Amaranthus. In addition bases are good forcing agents for Alisma as well as Sagittaria. The place and method of action of acids and bases as forcing agents have been variously interpreted. Fischer[22] found that the seeds of Sagittaria and some other water plants, which normally lie for years in water in a dormant stage, are readily forced by acids and bases. He believed that rapidly diffusing hydrogen and hydroxyl ions produce differences in potential thus arousing the dormant embryo.

It has since been shown that the embryos are not dormant and that the action is mainly upon the rather consistent gels of the coats leading to a mechanical weakening of these. This mechanical weakening is probably brought about by two means. As for gels quite generally acids and bases increase water absorption and thus lower the breaking strength in accordance with the law mentioned above. The coats consist of pectic or other materials rather readily hydrolyzed or otherwise decomposed by acids and bases and are mechanically weakened by such chemical transformations.

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[22] Fischer. Ber. Deutsch. Bot. Ges. **15**: 108. 1907.

Fischer found that the transfer of seeds of many water plants to foul water, in which abundant growth of bacteria and fungi occurred on the coats, aroused them from dormancy. He attributes the effect to acids or bases produced by the organism and acting upon the embryo. I am convinced that the rather slight forcing effect of organisms is due to enzymatic hydrolysis or decomposition of the coats and not to the acids or bases produced. In Alisma concentrations of acids, sufficient to give but slight forcing action, are too high to permit considerable growth of the embryo; and in Scirpus and Sparganium[21] no concentration of acid or base will force germination. We should except sulfuric acid (sp. gr. 1.84) which acts as a carbonizing agent and produces good germination of the former after nearly 3 hours' treatment but only abnormal germination in the latter after more than 24 hours' treatment. As we shall see later there is a well-established case in which the forcing action of the acid is upon the embryo. This is claimed as the point of action in light stimulated seeds where acids rather generally have the power of substituting for light, but this claim is entirely without critical evidence. In *Amaranthus retroflexus*, a light inhibited seed, the acid has its effect upon the coat. There is need of a critical study of the effect of acids on light sensitive seeds to learn what part of the seed is affected as well as the specific nature of the effect in each case.

As we have already mentioned, seeds of *Amaranthus retroflexus* may lie in moist soil for thirty or more years in a viable dormant condition. It is well to mention known and needed investigation that may aid in elucidating this phenomenon, for the findings for Amaranthus probably apply in a general way to seeds of black mustard, shepherd's purse, Lepidium and many others, that lie in the soil for years in a state of quiescence with the embryo partially or fully saturated with water. As aids to keeping the seeds of Amaranthus in a dormant condition in the soil there is the extra resistance to expansion offered by the soil and the power of these seeds to take on secondary dormancy, a matter discussed later. Such seeds are laid open to exhaustion of their foods by leaching and respiration. As is coming to be found the case for many seeds the coats of Amaranthus have a most efficient semi-permeable membrane. This is well shown by the fact that they can lie in a saturated solution of copper sulfate or $4N$ sodium chloride for weeks without marked injury. Examination will show whether leaching is further prevented by stored foods being held in the condensed condition. A thorough study of respiratory rate under conditions of temperature and moisture approaching those of soil dormancy will give an idea of food loss from this source and with leaching losses will indicate whether the longevity of these seeds in nature is determined by starvation. Life duration experiments such as Groves and I have conducted upon wheat may offer evidence as to whether these seeds will persist longer in dry storage or whether some process similar to Lepeschkin's postulated redispersal theory increases their longevity in the imbibed condition.

4. Of the seeds that are delayed in germination by seed or fruit coats reducing the oxygen supply below the minimum for germination, various species of Xanthium have[3] been most thoroughly studied. The character appears in both the upper and lower seeds of the bur but is more marked in the former. The seeds grow readily when the testas are removed. Increased oxygen pressure or hydrogen peroxide induce germination of the intact seeds. Under normal oxygen pressure high temperature acts as a forcing agent. This gives the odd phenomenon of two temperature minima for germination—one with coats removed and a much higher one with coats intact. In nature the lower seed generally germinates the year after ripening and the upper the following year. This regularity is often broken up by agencies that modify the testa or by high temperatures during the first summer.

Shull[24] in published articles and Denny in unpublished work have done much to clear up the mechanics of the behavior of Xanthium seeds toward oxygen. The naked embryos absorb much more oxygen from the air than embryos in the testas. Embryos in intact seeds show an increase in oxygen absorption with increase in the partial oxygen pressure of the atmosphere. *These facts establish the important point of increased consumption of oxygen under oxygen supplies favoring germination.* The naked embryos of both seeds have rather definite oxygen pressure minima for germination; this is considerably higher for the upper than for the lower seed and it falls for both as the temperature rises. *These facts explain in part both the differences in behavior*

[23] Crocker. Bot. Gaz. **42**: 265. 1906.

[24] Shull. Bot. Gaz. **52**: 453. 1911.

of the two seeds with the coats intact and the forcing action of high temperatures.[25] As time elapses, either in dry storage or in natural conditions, the coats become more permeable to oxygen; also the rate of oxygen absorption by the naked embryos falls. *These facts explain in part the timing of the delay in nature and the greater efficiency of increased oxygen pressure as a forcing agent at mid-winter than immediately after ripening.* In nature, however, frosts and many other conditions modify the testas; also the embryo may have its oxygen minimum lowered by sugar accumulation. For the latter, however, we have no evidence.

Upon the whole the mechanics of the dormancy and of its duration is fairly well elucidated for the seeds of the cocklebur.

Since the discovery of this method of delay in Xanthium, many other seeds, especially composites[26] and grasses, [27, 28] have been found to possess similar characters. It has been found that in unafter-ripened seeds of *Chloris ciliata* high temperature forces germination even in normal oxygen pressure as it does in the intact seeds of cocklebur; while the unafter-ripened seeds of wild oats, *Avena fatua*, and "rain barley" (barley ripening during rainy weather) germinate in normal oxygen pressure only at relatively low temperatures. As after-ripening progresses the minimum temperature falls in the first and the maximum rises in the last two. In *Chloris ciliata* light and certain salts will substitute for increased oxygen pressure. No experiments have been performed to elucidate most of these phenomena. It is not known for instance whether the vicarious action of light and salts upon Chloris is due to effects upon the coat increasing its permeability to oxygen, or upon the embryo, lowering its minimum oxygen pressure for germination as high temperature apparently does in the cocklebur. The disposition of the workers to philosophize rather than to outline and perform searching experiments has proved a great hindrance to progress.

It has been shown that the presence of glucose[29] may substitute for oxygen in the growth of certain plant organs as well as in the germination of seeds. It is possible that the amount of sugar present in seeds of the type now under discussion may determine their ability to germinate in limited oxygen supply and that agencies like light and salts act indirectly through sugar formation or carbohydrate hydrolysis. This and other possibilities need careful experimental study in this connection.

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[25] It is possible that permeability changes in the testa may also be induced by temperature changes.

[26] Becker. Inaug. Diss. Münster. 1911.

[27] Gassner. Jahrb. Ham. Wiss. Anstalten Beih. 1. **29**: 1911.

[28] Atwood. Bot. Gaz. **57**: 386. 1914.

[29] Lehmann. Jahrb. Wiss. Bot. **49**: 61. 1911.

Matters are made more difficult here by the fact that we do not know the exact method by which oxygen acts in determining growth and growth rate of organs of seed plants. Nabokich[30] gives it a multiple rôle. Directly, it stimulates growth as do salts and other substances. This function can be cared for by various other reagents. Next, it oxidizes what would otherwise be fatal products of anaerobic respiration and thereby makes continued life possible. Finally, he probably would not deny that indirectly at least it is important in releasing necessary energy for growth through normal respiration.

In connection with germination Becker[26] speaks of oxygen as having a catalytic function and Lehmann[31] connects it with the hydrolysis of proteins, thus furnishing substances necessary for the growth of the embryo. The assumptions of these authors are in great need of experimental evidence; they have such difficulties to meet as the facts that there is greatly increased oxygen consumption under increased oxygen pressure and that hydrolysis of proteins is extensive in certain seeds in oxygen-free[32] germinators. Kidd[7] finds that the narcotic concentration of carbon dioxide decreases with decreased oxygen pressure and believes that oxygen supply in seeds of the type under discussion operates indirectly through the anaesthetic action of carbon dioxide. Shull[33] is inclined to think that in the cocklebur the effect of oxygen is through increased respiration, for here the oxygen consumption increases greatly with increased partial pressure and with coat removal.

The unsolved problems here are among the most difficult and fundamental of those met in growth.[34]

Dude[35] finds that imbibed seeds *in vacuo* are soon killed by accumulation of toxic materials of intramolecular respiration. Of the five species studied the life duration in this condition ranged from about

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[30] Nabokich. Beih. Bot. Centralbl. **26**: 7. 1910.

[31] Lehmann. Biochem. Zeitschr. **50**: 388. 1913.

[32] Godlewski. Bull. Acad. Sci. Cracovie. **9**: 705. 1912.

[33] Shull. Bot. Gaz. **57**: 64. 1914.

[34] Höber. Phys. Chem. Zelle. u. Gewebe. 752–780. Berlin, 1914.

[35] Dude. Flora **92**: 203. 1903.

15 to 50 days. Mazé[36] found that seeds retain their vitality but a short time even under the considerable reduction in oxygen supply involved in water storage. Besides injury from toxic products of intramolecular respiration he found leaching of stored foods very extensive in some cases.

In the light of these facts the question occurs, Why is it that many seeds can have their germination inhibited by a sub-minimal oxygen supply without suffering from toxic products of partial anaerobic respiration? To answer this question we need a thorough study of the products of anaerobic and partial anaerobic respiration in cocklebur and other seeds of similar behavior.

In this connection we should also remember that seeds of various water plants[20] will withstand storage under water for years and with removal of coat restrictions will germinate fairly rapidly and grow rather extensively in total absence of oxygen. In these seeds anaerobic respiration apparently produces little if any poisonous products.

Shull[37] has observed that seeds of a number of wild forms, including both water and land plants, will withstand at least 4½ years' storage in water. The discrepancy between these results and those of Mazé is probably best explained by the fact that Shull was working with the seeds of wild plants while Mazé was dealing with cultivated species. A student in this field is impressed by the fact that through cultivation seeds have largely lost those characters that make for long dormancy and in general for success in the struggle under natural conditions.

Characters we have already pointed out for various seeds of wild plants will largely explain the findings of Shull. Hard coats, restricted swelling, partial anaerobic respiration that does not produce toxic materials, and perhaps finally the disposition of certain seeds to keep stored foods in the condensed rather than in the hydrolyzed condition, are all found in one seed or another showing dormancy and all reduce the rate of food exhaustion and tend to maintain a healthy condition of the embryo.

5. Turning to the fifth general method by which dormancy in seeds is secured, we find a situation not met in the previous classes: the embryo or a part of it fails to grow when naked and supplied with all the external conditions necessary for germination. It must go through a series of changes or after-ripen before germination can

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[36] Mazé. Ann. Ins. Pasteur, **14**: 350. 1900.

[37] Shull. Pl. World, **17**: 329. 1914.

occur. Of the seeds of this class *Crataegus mollis* has been most fully studied. Both the optimum conditions[38] for after-ripening and much of the nature of the changes[39] involved, have been worked out.

After-ripening occurs most rapidly at 5° C., but the structures surrounding the embryo influence greatly the rate of the process at this temperature—with the naked embryo 3 or 4 weeks are required, with the testa only intact 3 or 4 months, with both testa and stony carpel intact more than a year. Investigations indicate that the coats retard the after-ripening at least in large part by limiting the oxygen and water supply to the embryo. The possibility is not excluded, however, that they hold in some inhibiting substances.

Regarding the changes involved in the after-ripening of the seed Eckerson finds that in the dormant condition the hypocotyl is slightly basic or neutral to phenolphthalein, while the cotyledons, capable of immediate growth, are quite acid. As after-ripening progresses the hypocotyl becomes more and more acid. The acid reaction of the hypocotyl seems to produce general physical-chemical conditions favorable for water absorption, enzyme formation and action, and, through these, for growth. In accord with this certain acids greatly hasten after-ripening. Apple seeds behave qualitatively as Crataegus, but quantitatively the dormancy is much less persistent.

A study of several more representatives of this physiological type of seed is needed in order to know how generally the findings for the haw hold for the group.

There is no doubt that every one of the five types of dormancy we have described is represented by many different seeds. It is also likely that combinations of two or more types of these show up in a given seed. While in general the embryos are primarily responsible for the dormancy in the first and fifth types and the coats for the second, third and fourth types, every one shows a more or less complex interaction between the coats and the embryos.

Secondary Dormancy.—It is a rather generally observed fact that some seeds capable of immediate germination can be thrown into a secondary dormancy by a period in a germinator lacking some one condition necessary for germination or involving a substance inhibiting germination or one hardening the colloids of the coats. A few cases may be cited. Gassner[27] produced dormancy in the light requiring seeds of *Chloris ciliata* by a period at 20° C. in a dark germinator; Kinzel[40] in the dark requiring seeds of *Nigella sativa* by a period in an illuminated chamber at 20° C.; Crocker and Harrington[41] in Johnson grass (*Sorghum halepense* L.) and Davis and Crocker[21] in *Amaranthus retroflexus* by a time in the germinator at subminimal temperatures; Kidd in white mustard by exposure of imbibed seeds to sufficient partial pressure of carbon dioxide and Crocker and Davis[20] in the acid treated seeds of *Alisma plantago* by treatment with almost any concentration of copper sulfate. In all these cases except Nigella the authors find that the modification is in the seed coats and that the embryos are at no time dormant. This interpretation for Nigella accords with the known facts quite as well as Kinzel's interpretation. Except for Nigella, on which experiments relative to this point have not been made, removal or rupture of the coats gives immediate and vigorous germination and in white mustard drying produces the same effect.

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[38] Davis and Rose. Bot. Gaz. **54**: 49. 1912.

[39] Eckerson. Bot. Gaz. **55**: 286. 1913.

Some evidence has been gained regarding the nature of the changes involved in dormancy production, but there is need of much more work on this point. In some cases it involves such simple reversible processes as the stoppage of free gaseous exchange by the filling of capillary spaces with water.[42] In other cases pectic or other gel-like materials fill the spaces.[43] In *Alisma plantago* the copper ions harden the coat colloids, increasing their breaking strength and rendering impossible the further osmotic and imbibitional swelling of the embryo. In white mustard Kidd believes the carbon dioxide lowers the permeability of the coat to gases, thus hindering the free elimination of carbon dioxide and absorption of oxygen. The carbon dioxide narcotizes the embryo and the reduced oxygen supply is important in lowering the amount of carbon dioxide necessary for narcosis. Kidd[7] has shown that the carbon dioxide content of soils is sometimes sufficient to induce dormancy in white mustard. This factor is, therefore, operative in natural conditions. It is probably limited in its significance to relatively few seeds, for many seeds (cabbage, onion, barley, beans, and peas) cannot be thrown into dormancy by any partial pressure of carbon dioxide.

In *Amaranthus retroflexus*[21] I believe dormancy production is the

[40] Kinzel. Ber. Deutsch. Bot. Ges. **25**: 269. 1907.

[41] Unpublished Work at the Seed Laboratory of U. S. Department of Agriculture.

[42] Haberlandt. Bot. Jhrb. **3**: 857, 1875.

[43] Windisch. Wochenschrift Brauerei, **22**: 89. 1905.

counterpart of after-ripening and involves partially reversible changes in the colloids of the seed coats in which hysteresis is important. Hysteresis is very common in colloids and involves the lagging of certain secondary changes behind the primary change that causes them. In *Amaranthus retroflexus* seeds, a month or more of dry storage is necessary for after-ripening. The after-ripening seems to consist merely in a lowering of the elasticity and breaking strength of the colloids of the coats, rendering the force of the expanding contents capable of rupturing them. On the basis of data we need not cite here I interpret after-ripening and dormancy production in these seeds as follows. Upon drying, the colloids of the coats slowly take on new characters which lag a month or more behind the drying and give them much lower elasticity and breaking strength when again soaked up. *This is after-ripening.* When soaked up the coats do not immediately take on that character stipulated by high water content; but if the seeds, in a state of saturation, are prevented from breaking the coats, the old strength is gradually attained and dormancy secured. It is possible that Kidd in white mustard was dealing with hysteretic changes involving permeability characters and that carbon dioxide was acting merely as an inhibitor to germination allowing these time changes to occur rather than acting directly upon the coats as he thinks. Undoubtedly other changes in the coat of non-reversible type are sometimes involved in producing dormancy.

While in the cases of induced dormancy, cited above the main change is in the coats, these changes act in every case through some character of the embryo or other seed or fruit organs—the force with which they expand, their capacity to be narcotized by carbon dioxide, etc. Conditions inducing dormancy by coat changes must simultaneously bring about important changes in the seed contents; such as hydrolysis and condensation of stored foods and enzyme formations. These, however, play no rôle or only a minor rôle in dormancy induction. Finally it is likely that lasting dormancy may be induced in which the significant changes are in the embryo. Indeed this is assumed to be the general situation by certain workers who are unduly imbued with a certain type of ultravitalism that prevents a thorough physical and chemical analysis of the problem.

The biological significance of this secondarily induced dormancy is clear. It throws many seeds into a condition of quiescence in nature from which they may be aroused only by a marked change in conditions on one hand or a slow process of decay of the coats themselves on the other. This gives seeds ready for germination at every break in vegetation—a matter of great importance in the struggle among plants. Induction of dormancy in seeds capable of germination also throws much light upon a phenomenon very common in nature—the primary dormancy taken on by seeds of non-viviparous plants with the approach of ripening. As Guppy and Kidd have emphasized, vivipary is generally connected with absence of seed coats or with poorly developed ones. Well-developed coats play a dominant part in primary as well as in secondary dormancy; on the other hand we have already shown that primary dormancy is sometimes due in the main to the embryo and is only lengthened by the coats.

Forcing Agents

The effect of forcing agents upon dormant seeds is a topic deserving much attention, but time will compel us to limit it to a few general statements. Already a number of references have been made to certain forcing agents.

Acids and bases are frequently found effective through a physical or chemical modification of the colloids of the coats, while in the haw acids apparently force germination by changing the reaction of the embryo.

Freezing or freezing and thawing[44, 45] are means of forcing and probably are of very great significance in nature. In general the work in this line has not been directed at the mechanism involved and throws little light upon the part of the seed effectively modified as well as the nature of the modification. Low temperature, but not freezing or freezing and thawing, hastens the after-ripening in the embryo of Crataegus. Evidence indicates, however, that the beneficial effects of freezing are often through coat changes.

The forcing action of salts[27, 31] is frequently reported, but here again the studies are not directed at the mechanism involved, although many writers make the gratuitous assumption that their effects are nutritive or stimulative to the embryo. They will have to meet the rather general existence of non-living semi-permeable coat membranes that permit little or no entrance of salts. It may be found that salts are often effective through a modification of the colloids of seed coats.

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[44] Kinzel. Frost und Licht als beeinflussende Kräfte bei der Samenkeimung. Ulmer, Stuttgart, 1913.

[45] Pammel and Lummis. Proc. Soc. Prom. Agr. Sci. **24**: 89. 1903.

Soil as a substratum[27, 31, 48] frequently forces the germination of dormant seeds. It also produces secondary dormancy. Here a number of reagents may play a part, each acting either upon the coat or upon living structures: changed oxygen supply, salts, acids, bases, and other substances. Kidd has shown that mustard seeds are thrown into secondary dormancy by the high carbon dioxide content of certain soils. Other narcotizing and inhibiting substances may also be present in the soil.

High temperature[23] is frequently effective. In some cases it acts through coat restrictions to oxygen or water absorption. These restrictions give a germinative temperature minimum far above that of the naked embryo. Independent of coat restrictions, however, the minimum temperature for the germination of seeds of many wild forms is very high, being near 20° C. This embryo character accentuated by coats plays a very great rôle in dormancy in nature. Alternation of temperatures[27, 46] is also an effective means of forcing many seeds. It is extensively used for practical testing in the seed laboratory of the U. S. Bureau of Plant Industry. The method of action of alternating temperatures is not known.

Finally light[46] is an important condition in determining dormancy: it forces the germination of many seeds that would otherwise be dormant and inhibits many that would grow in darkness, while the greater number of seeds are indifferent to it. In certain seeds Kinzel has connected the effect of light with mobilization of reserved materials while Lehmann and Ottenwälder[48, 49] on the basis of inferential and philosophical considerations give it a rôle in protein hydrolysis. The assumption that it is always effective through modification of living substances alone is quite gratuitous. It may modify either the living structures or the coats, each in a variety of ways. It must be recognized that the latter are in the more exposed position. There is great need of a scrutinizing chemical-physical study of the effect of light upon seed germination.

We must point out a biologically significant matter concerning light requiring seeds: in the soil they lack this one condition for germination and are held in a dormant state. Some seeds (Nicotiana and others), belonging to this class, hold their vitality for years in the soil.[49]

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[46] Pickholz. Zeitschr. Land. Versuchs Oesterr. **14**: 124. 1811.

[47] Lehmann and Ottenwälder. Zeitschr. Bot. **5**: 337. 1913.

[48] Ottenwälder. Zeitschr. Bot. **6**: 785. 1914.

While we are discussing light as a forcing agent, let us revert to certain points in the classification we have used in this paper. The question may justly be raised, Why not classify lack of light as one of the methods of securing dormancy in seeds, just as was done for oxygen, rather than treat it as a forcing agent? This question must be answered by repeating that the two general topics are closely interrelated and that while the present classification has virtue in convenience of discussion and in giving aim to future research it must be subject to modification with advance in knowledge. Again free oxygen is generally spoken of as an essential condition for growth of organs of flowering plants while light is generally merely a formative condition and only indirectly necessary through food supply. True, later work has somewhat modified the clearness of this distinction. Free oxygen is not necessary for considerable growth in seeds of various water plants and can be vicariously displaced by other conditions for limited growth in certain organs of other flowering plants. Even if its main function in maintaining continuous growth is the oxidation of toxic products of intramolecular respiration, it stands in a much more immediate essential relation to growth than does light. Moreover we do not know how general and immediate a relation oxygen holds to continuous growth through supply of necessary energy by normal respiration nor how generally its so-called stimulative effects can be temporarily displaced by other conditions. For light-sensitive seeds, light has been found capable of displacement in one after another until very recently the most persistent case, seeds of Gesneriaceae,[50] have yielded to experimentation. I am much inclined to believe that future investigation will show that light acts through the elimination of one of the other five dormancy producing factors mentioned above rather than in such a fundamental and essential relation as oxygen.

SUMMARY

In closing let us consider a few of the more important general bearings of the facts treated in this paper.

1. Dormancy in seeds results generally from the inhibition of one

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[49] Duvel and Goss. Unpublished Work on buried seeds referred to in the Introduction.

[50] Gassner. Zeitschr. Bot. 9: 609. 1915.

or more of the processes preceding or accompanying germination. The problems are becoming questions of the conditions for growth of the embryo and the fundamental changes occurring in the embryo with the beginnings of germination on one hand: and a study of the physical characters (permeability and breaking strength) of the colloids of the seed coats as affected by aging, various conditions and reagents, upon the other.

2. Seed coats have a surprisingly important rôle to play in both primary and secondary dormancy. Often they are of such colloidal nature as to be modified by even very low concentrations of a variety of reagents thus permitting the growth of the embryo. In the past such results have been interpreted frequently and wrongly as stimulus responses.

3. Regarding conditions for germination of seeds, the recent trend is toward the need of certain general physical conditions and away from the need of specific chemical stimuli, or even chemical stimuli at all. The same change in view is occurring in reference to germination of pollen.[51, 52] We apparently have here a generalized physiology in contrast to the situation in the organs of more highly differentiated organisms. In mammals for instance dormancy of an organ as well as its rate and course of development are often determined by specific substances from the great number of highly specialized glands. Even if Kidd's conclusion is correct, that carbon dioxide often produces dormancy by narcotizing seeds, we have the action of a general product of metabolism rather than a specific material.

4. After-ripening of seeds, or the changes occurring during dormancy and finally making germination possible, may involve growth of a rudimentary embryo, fundamental chemical changes in an otherwise mature embryo, or chemical changes in the coats. In after-ripening there is often a complex interrelation between coat and embryo changes.

5. Problems in dormancy lend themselves beautifully to the mechanistic attack.

6. Finally, I must state that the dominantly mechanistic interpretation I have given dormancy in seeds is not that held by all or perhaps even a majority of the workers in this field; but this viewpoint has recently made great advances possible and promises much for the future.

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[51] Tokugawa. Journ. Coll. Sci. Tokyo, **35**: 1. 1914.

[52] Martin. Bot. Gaz. **56**: 112. 1913.

THE PERIODICITY OF FRESHWATER ALGAE*

Edgar Nelson Transeau

The normal life of a freshwater alga such as a Spirogyra consists of a period of germination of spores, a period of growth and development, and a period of sexual or asexual reproduction, commonly followed by a period of dormancy. An Oedogonium has in addition to these periods one or more phases during the vegetative period when zoospores are produced. Other green algae have part or all of these several periods. Consequently if we follow the changes in the algae occurring in a given pond or stream throughout the year, we find a rather regular succession of life phases for each of the species present. Since the life cycles of the different species vary in their duration, we also find an orderly sequence of appearing and disappearing species.

To discover the causes underlying these periodic changes, efforts have been directed in two rather distinct lines of investigation: (1) The observation of algae under laboratory conditions, and (2) The observation of algae under natural conditions.

To the first class belong the investigations of Klebs, Artari, Benecke and Danforth. These experiments have given us a considerable body of information concerning the effects of light, temperature, concentration and the chemical nature of the medium. The results of the experiments with variations in light and temperature as factors in accelerating or retarding vegetative and reproductive activities are for the most part qualitative. They still await a quantitative statement of their relations. The experiments with concentration and chemical composition of the medium not only show very inharmonious results, but the conclusions to which they have led are scarcely applicable to the explanation of the periodicity of algae in nature, since the concentrations used are many times the concentrations of our natural waters, and many of the substances used do not occur in our pond and stream solutions. These results may be of great interest in cell physiology, but they do not appear to be applicable to the conditions out of doors.

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The second source of information is the study of algae in the field. Comere studied the algae of the vicinity of Toulouse, France. In addition to a classification of the habitat groups of algae, he classified the local species into seasonal groups on the basis of their times of reproduction alone. Fritsch and West in England have contributed a number of papers dealing with the frequency and reproductive activities of the algae in a number of pools and ponds. Some of the records of Fritsch cover a period of five years and are correspondingly valuable. In this country Copeland made a two-year study of the periodicity of the Spirogyras, and Brown published some records of the occurrence of algae in southern Indiana.

As a result of these field studies there have developed two extreme points of view: Copeland came to the conclusion that his observations "offer overwhelming evidence in support of the view that the phenomenon of conjugation results not so much from external as from internal conditions," and "that Spirogyra has definite periods of growth and reproductive activity"; Fritsch on the other hand assumes to account for all the phenomena of germination, vegetative development and reproduction on the basis of changes in the environment. He emphasizes especially the effects of temperature, light, and concentration of medium, although he made no determinations of the actual concentration of the waters with which he dealt.

The present paper is based on seven and a half years continuous records of the algal conditions in central Illinois. About 3,000 collections have been analyzed. Particular attention has been given to the Zygnemales, the Oedogoniales, and the other filamentous forms. As a result there are notes on the occurrence of over three hundred species, and sufficient data to establish the periodicity curve of about half that number. Aplanospores, zoöspores, zygospores and oöspores have been recorded more than a thousand times in the course of the work.

Our algae fall with few exceptions into six natural groups, based on the time of their germination, vegetative development, reproduction and dormancy (Fig. 1).

1. The *Winter Annuals* begin their vegetative activities in the autumn, increase these activities up to the time the ponds are frozen over, and pass the winter under the ice. They may develop further during protracted winter thaws and may even fruit. Their reproductive activities culminate in March and April, although sexual spores

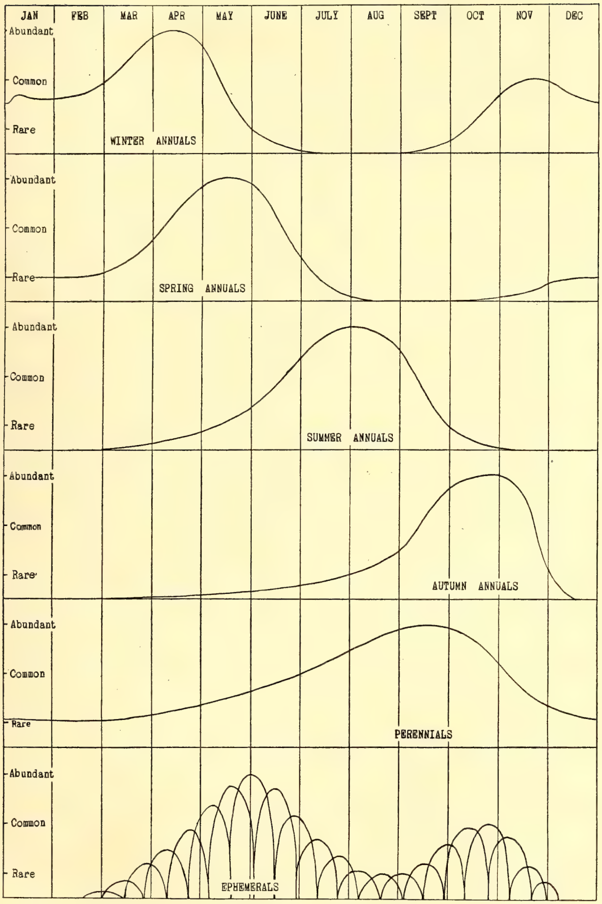

FIG. I. Curves of frequency of the six ecological groups of algae.

may be produced at any time from November to April. Zoöspores are formed during the period of vegetative development, and aplanospores and akinetes during the period of decline. Prominent among the winter annuals are: *Conferva minor* Klebs, *C. bombycina* Agardh, *C. utriculosa* Kütz., *Vaucheria geminata* (Vauch.) DC., *V. sessilis* (Vauch) DC., *Draparnaldia plumosa* (Vauch.) Ag., *Tetraspora lubrica* (Roth) Ag., *Stigeoclonium lubricum varians* (Hazen) Collins, *Gomphonema angustatum* Grun., *G. acuminatum* Ehrb., *Spirogyra tenuissima* (Hass) Kutz., *S. inflata* (Vauch) Kütz. and *Oedogonium rufescens* Wittr.

2. The *Spring Annuals* begin their vegetative period in late autumn or early spring, attain their maximum development and the reproductive stage during May. This is by far the largest of the seasonal groups. Among the common forms are: *Zygnema stellinum* (Müll.) Ag., *Z. leiospermum* De Bary, *Z. insigne* (Hass.) Kütz., *Spirogyra catenaeformis* (Hass.) Kütz., *S. varians* (Hass.) Kütz., *S. protecta* Wood, *Mougeotia scalaris* Hass., *M. robusta* (DeBary) Wittr., *Oedogonium echinospermum* A. Braun, *Oe. multisporum* Wood, *Oe. suecicum* Wittr., *Bulbochaete crassiuscula* Nordst., *Debarya decussata* Transeau, *Vaucheria hamata* (Vauch.) DC., *Draparnaldia Ravenellii*, Wolle, *Coleochaete scutata* Breb., and *Herpesteiron confervicola* Naeg.

3. The *Summer Annuals* germinate in the spring. The greatest reproductive activities occur in July and August. Zoöspores are most frequent in spring and early summer. Aplanospores develop mostly in August and September. Among the common summer annuals are *Oe. praticolum* Transeau, *Oe. varians* Wittr., *Oe. vaucherii* (LeCl.) Braun, *Calothrix stagnalis* Gomont, *Spirogyra ellipsospora* Transeau, *S. nitida* (Dillw.) Link, *S. irregularis* Nägeli, *S. setiformis* (Roth) Kütz., *Mougeotia sphaerocarpa* Wolle, and *Cylindrocapsa geminella minor* Hansgirg.

4. The *Autumn Annuals*. These species begin their vegetative development in late spring, increase through the summer and have their maximum abundance in the autumn. Sexual reproduction if present, occurs mostly in September and October. This is a comparatively small group, and includes *Rivularia natans* (Hedw.) Welw., *Oedogonium capilliforme* Kütz., *Oe. macrandrium* Wittr., *Oe. obtruncatum* Wittr., and *Oe. crassum amplum* (M. & W.) Hirn.

5. The *Perennials*. This group includes species whose vegetative cycle may be or is continuous from year to year, in permanent streams and ponds. Reproduction may occur at any time in some of the

forms, but in general is more abundant during May and June. Some local examples of perennials are *Rhizoclonium hieroglyphicum* (Ag.) Kütz., *R. fontanum* Kütz., *Cladophora glomerata* (L.) Kütz., *C. fracta* (Dillw.) Kütz., *Pithophora varia* Wille, *Tolypothrix tenuis* Kütz., *Hyalotheca dissiliens* Breb., *Desmidium swartzii* Ag., *Mougeotia genuflexa* (Dillw.) Ag. (in permanent ponds) and *Zygnema pectinatum* (Vauch) Ag. (in permanent ponds).

6. The *Ephemerals* are species having vegetative cycles of a few days or at most a few weeks' duration. Generations succeed one another rapidly during the periods of favorable conditions. These species are mostly plankton and soil algae. Zoöspores are the usual means of reproduction, and aplanospores and akinetes carry them over the unfavorable seasons. Some examples of ephemerals are *Botrydium Walrothii* Kütz., *Ineffigiata neglecta* W. & G. S. West, *Pediastrum Boryanum* (Turp.) Meneg., *Scenedesmus quadricauda* (Turp.) Kütz. and *S. bijuga* (Turp.) Wittr.

The recognition of these classes is of ecological interest because of the greater ease of description of the life habits of a particular species. In the present connection it is of importance because the finding of great irregularity in the behavior of some form or group of forms may suggest the causes correlated with the irregularity, or a method of experimentation which will lead to the causes. I believe that the most prolific source of contradictory results among experimenters has been a lack of knowledge of the normal periodicity of the algae in the field, and the failure to appreciate the importance of the time of germination and the vegetative age of the materials used in the experiments. I have hoped throughout this study of the behavior of algae in the field, that it might furnish new points of departure for experimentation.

The other results of these field observations may be conveniently presented under four heads: (1) The time and conditions of germination, (2) the vegetative cycle, (3) the reproductive period in relation to external conditions, and (4) the method of reproduction in relation to heredity and external conditions.

Germination.—The vast majority of zygospores, oöspores and aplanospores germinate in the spring. But the period during which the spores of a particular species germinate is often extended over several weeks. This is indicated both by field observations and laboratory experiments. Further, there is some germination going on at all times of the year, a secondary maximum occurring in September and October. In the years with heavy autumn rains this is especially marked. The temperature as a control of the germination of algal spores has probably been overestimated. The spores of many species will germinate at all the ordinary water temperatures. There is still less reason for speaking of the germination as coincident with "rising" or "falling temperatures." The data in hand point rather to the conclusion that all those factors which contribute to the germination of seeds are also operative here. Increased oxygen content of the water, increased mineral content, and induced changes in the permeability of the spore coats are probably the controlling factors, the speed of germination being retarded or accelerated by the temperature.

Length of the Vegetative Cycle.—So far as I am aware this has received scant attention among experimenters. In some forms like Pithophora and Vaucheria it is probably of no consequence for they may be induced to fruit immediately upon germination. But in other forms like the Zygnemales and Oedogoniales it is probably of vital importance in the interpretation of experimental results. In these forms the vegetative cycle is a period of the formation of the filament by cell division, and the period of accumulation of nutrient and other materials. During this period photosynthesis, proteinsynthesis and assimilation are active on the one side, while respiration, accumulation and growth are active on the other. Field observations and experimentation indicate that this must go on for a certain length of time before reproduction is possible. They also indicate that the speed of the metabolic processes must attain a certain minimum rate or reproduction fails to close the vegetative cycle. A number of factors may limit the speed of metabolism; light of low intensity is obviously one that operates so effectively, that algae in shaded portions of streams may never reproduce, or even be able to maintain more than a temporary existence. It is probable that excessively high temperature may also prevent the normal development of the algae. Higher temperatures accelerate and lower temperatures retard the completion of the vegetative cycle. These results are just as clearly indicated by field observations as by experiment. The amounts of available oxygen and carbon dioxide in the water also act as limiting factors to these processes.

The length of the vegetative cycle is so regular from year to year, that, given the conditions, it is not difficult to predict the order in which the species occurring in a particular pond will fruit. If we take one of the larger genera like Oedogonium, Spirogyra or Zygnema, we find a remarkably regular annual succession of species. In a general way there is an evident correlation between the length of the vegetative phase and size of the cells. In the case of the Spirogyras I have attempted to analyze this relationship more definitely. If we arrange the species of this genus in the order of their time of fruiting, we can compare this order with that of the cell diameters, cell volume, and total cell surface. There is no very close coincidence between the order of any of these dimensions and the order of lengths of the vegetative cycle. When, however, the total surface is divided by the volume and these quotients are compared, *i. e.*, when the specific surfaces of the cells are arranged in the order of their magnitude the correlation is very striking, and we are justified in assuming

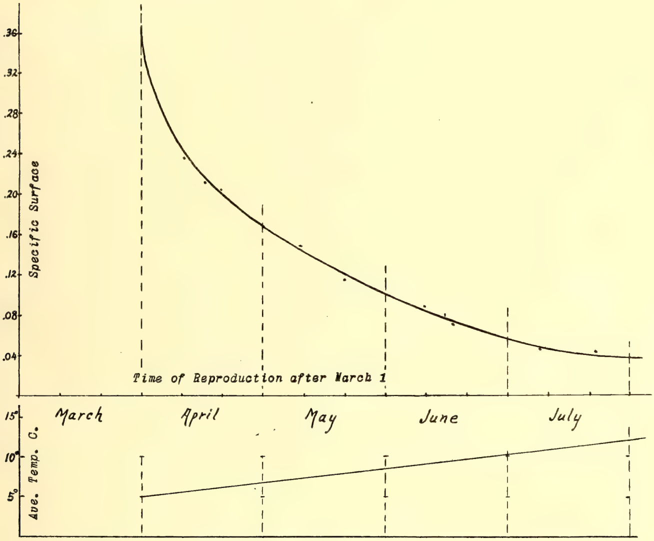

FIG. 2. Above is the curve for the specific surface against the time from March 1 to fruiting. Below is the curve for the temperature against the time to fruiting.

that the length of the vegetative cycle is an inverse function of the specific surface of the cells. This may be explainable on the basis that the processes of absorption, photosynthesis, proteinsynthesis, and assimilation are limited by the cell surface, while the capacity for accumulation is limited by the volume. Hence other conditions being equal in the cells with the largest specific surfaces we might expect the most rapid approach to maturity (Fig. 2). It has been shown that plant processes are accelerated by temperature, being doubled by a rise of ten degrees. Since the temperature gradually rises during this period a correction must accordingly be made. Nearly all the species germinate in March and for those that fruit in about thirty days the average temperature is about 5° C. For those that fruit in July the average temperature of the whole vegetative cycle is about 12° C. We have then three variables the lengths of the vegetative period, the specific surfaces, and the temperatures. The interrelations of these three variables may be represented by the empirical formula

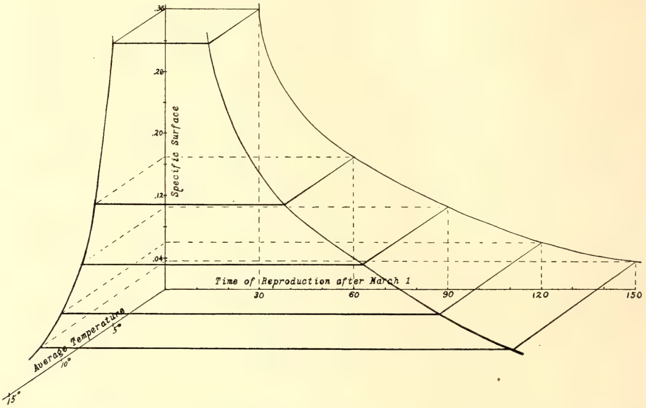

FIG. 3. Curve for the three variables: specific surface, temperature, and time to fruiting.

$$M = \frac{c}{\sigma \frac{t}{10}},$$

in which M is the length of vegetative period, c is a constant, σ is the specific surface, and t is the average temperature. The constant has been found to have a value of 6.5, and the formula may be written $\sigma t M = 65$. Figure 3 presents the interrelations of the three variables. Table I presents some of the average dimensions and other figures upon which the curve was constructed.

Table I

In this table the species of Spirogyra are arranged in the approximate order of their fruiting, when germination occurs about March 1. Following are the average dimensions; time of reproduction; approximate temperature of the whole vegetative period; the specific surface; the order by diameters; order by volumes; order by specific surfaces; finally the calculated length of the vegetative period, using the empirical formula $\sigma t M = 65$.

Spirogyra	Average Dimen.	Reprod.	Temp. Veg. Per.	σ	Order Diam.	Order Vol.	Order σ	M ($\sigma t M = 65$)
								days
tenuissima.......	12 × 120	Apr.	5° C.	.350	1	2	25	37
inflata..........	18 × 140	Apr.	5°	.236	2	4	24	55
communis.......	22 × 65	Apr.	5°	.212	3	1	23	61
catenaeformis....	26 × 70	May	6°	.182	4	3	22	59
Juergensii.......	28 × 90	May	6°	.165	5	5	21	67
Weberi..........	28 × 150	May	6°	.156	5	12	20	69
Grevilleana......	30 × 90	May	6°	.155	6	7	19	70
Lagerheimii......	30 ¼ 120	May	6°	.150	6	9	18	72
varians.........	36 × 75	May	6°	.138	8	6	17	78
areolata.........	34 × 200	May	7°	.127	7	17	16	73
fallax...........	36 × 150	May	7°	.124	8	13	15	74
protecta.........	36 × 200	May	7°	.121	8	19	14	76
decimina........	40 × 100	May	7°	.120	10	10	13	77
reflexa..........	38 × 150	May	7°	.118	9	16	12	78
fluviatilis........	40 × 140	May	7°	.114	10	15	11	81
porticalis........	42 × 130	May	8°	.110	11	14	10	74
condensata......	52 × 60	May	8°	.110	13	8	10	74
dubia...........	46 × 90	June	8°	.104	12	11	9	78
stictica..........	46 × 240	June	8°	.095	12	20	8	85
novae-angliae....	55 × 220	June	9°	.082	14	21	7	88
diluta..........	78 × 90	June	9°	.073	16	18	6	98
majuscula.......	60 × 300	June	9°	.073	15	24	5	98
submaxima......	86 × 150	June	10°	.059	17	22	4	110
maxima.........	120 × 120	June	10°	.050	19	23	3	130
setiformis.......	100 × 200	July	11°	.050	18	25	3	130
crassa..........	150 × 150	July	11°	.040	21	26	2	147
ellipsospora......	140 × 250	July	12°	.036	20	27	1	150

Additional evidence is given for the correctness of this supposition in the fact that in several cases for which I have sufficient data concerning large and small forms of the same species, the dates of fruiting are here also in harmony with the idea that the length of the vegetative cycle is a function of the specific surface.

Reproduction.—It is evident from the foregoing that in the Zygnemales and probably the Oedogoniales that the length of the vegetative period is the important period for study and experimentation, while the period of sexual reproduction is the supplement to this period. In some of the other orders of algae this period of accumulation seems to be less important and reproduction may be induced at all times by changes in the environment. Zoöspore production is induced in nature by a sudden rise of temperature, and by changes in light. The effect of nutrients and other chemical substances on reproduction cannot be determined from field observations. But when the collections from waters of sand and shale regions are compared with those of the prairie there can be no doubt that the number of fruiting species is very small. The length of the time required for the formation of the gametes, the union of the gametes and the formation of the spore is undoubtedly shortened by a rise in temperature, and lengthened by a lowering of the temperature.

In these more specialized groups, then, the environmental factors operate in general to accelerate, or retard or even inhibit the process of maturing.

One factor, however, deserves especial attention because of the fact that it has been emphasized so many times in connection with the reproduction of algae in nature: the concentration of the water. This matter of concentration has been appealed to both by those who think of it in terms of osmotic pressure, and those who think of it as increasing the mineral nutrients. The same idea is sometimes expressed in terms of the water levels. In all these cases the assumption has been made that the lowering of the water levels in a pond or pool results in the concentration of the pond solution. Three years ago I pointed out that, in general, algae fruit more abundantly during high-water periods than during low-water periods. This statement can be made on a still surer basis to-day than at that time. Now some of Klebs's experiments have shown that the fruiting of algae may be accelerated by increasing the concentration. Perhaps it is these results coupled with the experience of seeing waters in aquaria become concentrated through evaporation that has led to belief in the correlation between concentration and fruiting.

Since my figures ran contrary to this idea that algae fruit when the ponds are drying up, I made periodic determinations of the freezing points during 1913, 1914 and the spring of 1915. These determinations number somewhat more than two hundred, and it has been possible to see the effects of torrential rains, showers, and the most prolonged droughts known in central Illinois. In general the results indicate that the highest concentrations coincide with the periods of greatest rainfall and higher water levels, and the periods of low concentration are coincident with low water levels and drought. This result may be readily explained since the rains bring in the soluble salts from the upper layers of the soil. But the rains also bring in silt, clay and suspensoids. These require days and weeks to settle but meanwhile they have exposed an enormous surface for adsorption to all parts of the pond solution, and when they finally settle to the bottom they take nearly all the soluble salts with them. Indeed in the autumn of 1913 the Beckmann thermometer scarcely distinguished between distilled water and certain pond solutions. Since the rains in Illinois are mostly in the spring and autumn there are two annual maxima of concentration, one coinciding with the beginning of the spring rains and a lesser one coinciding with the beginning of the autumn rains. In late summer and late winter the concentrations reach their minima. I wish to be clearly understood to apply this statement only to pools, ponds, and streams fed by surface run-off. I have a series of determinations for the underground water of a well and in this case the water reaches its greatest concentration in late summer. This harmonizes with the numerous analyses of our large streams fed by springs and underground water, in which it has been clearly shown that the water concentrates in late summer.

The amount of concentration is also of interest to those who have relied on the osmotic pressure as a factor in producing the reproductive phase. The waters of the ponds and streams which I studied had an osmotic pressure of from one-tenth to one four-hundredth of the osmotic pressure of the cell sap. In the case of the waters, the depression of the freezing point varied from 0.002°–0.043°, in the case of the algae it varied between 0.4° and 0.9°. So that at most the osmotic pressure outside the cell is but a small fraction of the pressure on the inside of the cell.

To return then to my observations on the fruiting of algae as coincident with the periods of high water, these are also the periods of high concentration. And if these results need be in harmony with the concentration experiments, they are. But it is doubtful whether they need bear any relation to these experiments, for the concentration used is not ordinarily attained or even approached in nature.

The Method of Reproduction.—I have already spoken of the seemingly slight changes in conditions which initiate the production of zoöspores. In nature they are for the most part produced during the earlier part of the vegetative period. I wish to speak more particularly regarding the reproduction in the Zygnemales. As is well known conjugation may occur between cells of the same filament (lateral conjugation), or between cells of different filaments (scalariform conjugation), or they may produce aplanospores without conjugation. This last method I have found to be much more common than has been hitherto supposed. The question arises, are these different methods of reproduction controlled by environmental factors or do they indicate different hereditary strains of the species? Field observations point to the conclusion that they are hereditary qualities rather than effects of the medium in which they grow. The evidence may be summarized as follows:

1. All three modes of reproduction, indicated above, may be found in adjacent cells of the same filaments; (2) the three forms of reproduction may occur simultaneously in different species making up a single small mass of algae; (3) in certain ponds certain species have been found annually, producing only aplanospores, in other similar ponds producing only zygospores or both; (4) in several species of Zygnemales the conjugation may be almost entirely lateral in one mass collected and just as completely scalariform in another. Without a knowledge of the field conditions these last two proofs may seem as good evidence for the other side, but knowing the conditions under which these differences occur I think no one would question the interpretation given here.

Summary.—In conclusion, I wish to summarize the more important facts brought together in this study of algal periodicity.

1. Although algae germinate, develop vegetatively, and produce spores throughout the year, they may be conveniently grouped, on the basis of their *complete* life histories, into winter annuals, spring annuals, summer annuals, autumn annuals, ephemerals, and perennials.

2. The contradictory results of experimenters on the production of spores in the algae may be due to the neglect of the normal periodicity and vegetative age of the algae used in the experiments.

3. Four distinct periods may be recognized in the life history of most fresh-water algae: germination, vegetative development, reproduction, and dormancy.

4. The great majority of zygospores, oöspores, and aplanospores germinate in the spring. There is germination going on however at all times, and a secondary maximum occurs in the autumn.

5. The factors involved in the germination of the spores are probably as numerous as those initiating germination in seeds. The importance of temperature has probably been overestimated.

6. The length of the vegetative period in some forms is quite indefinite. In the Zygnemales and Oedogoniales it probably has a definite length under normal conditions.

7. Temperature, light intensity, concentration and mineral content of the water accelerate or retard the approach of the reproductive period.

8. The normal length of the vegetative cycle in Spirogyra is an inverse function of the specific surface of the cells. This is possibly also true in Zygnema and Oedogonium.

9. The normal length of the vegetative cycle in species of Spirogyra is approximately equal to a constant (65) divided by the specific surface times the temperature.

10. The concentrations of the waters in pools, ponds and surface streams attain their maxima in early spring and autumn, corresponding in general with the periods of heavier rainfall.

11. The lowest concentrations occur in late winter, and at the end of a prolonged drought in summer.

12. The periods of most abundant fruiting of algae correspond with the periods of high water levels.

13. The concentration of natural waters at their maximum is so small in comparison with the concentrations of the cell sap that it is doubtful whether it is of any significance in initiating reproduction.

14. In the Zygnemales, lateral conjugation, scalariform conjugation and aplanospore production appear to be hereditary tendencies rather than the result of environmental conditions.

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THE MORPHOLOGY AND AFFINITIES OF GNETUM

Walter P. Thompson

1. Introduction

The Gnetales have been called the lure and the despair of the morphologist. They are alluring because they promise to give a solution to the morphologist's great problem, the origin of the Angiosperms. They are a despair in that heretofore, in spite of many efforts, no one has been able either to establish convincingly or to disprove their Angiospermic connection. Some botanists maintain that they occupy a sort of intermediate position between Angiosperms and Gymnosperms; others believe that they represent a line of evolution which developed parallel to the Angiospermic line from the same ancestral group; others again deny all relationship between the two groups, believing that the undoubted points of resemblance have been independently acquired. Nor is there agreement in regard to their Gymnospermic connection. While most morphologists are agreed that they are the highest of the Gymnosperms some believe that they have been derived from the Bennetitales and others that they have come from the Coniferales.

An obvious contribution toward a solution of these problems would be a thorough investigation of the essential morphology of the genus Gnetum about which our information is very meager. In addition to its own interest and its bearing on general problems of Gnetalean and Angiospermic affinities, it should throw light on other problems such as the morphology of the gametophytic structures and endosperm of Angiosperms. Undoubtedly the morphology of Gnetum would have been thoroughly investigated long ago but for the great difficulty in securing proper material. With our present accumulation of knowledge in respect to the gametophytic conditions and endosperm of almost every other living genus of Gymnosperms and of representative forms from the whole Angiospermic series, the need of the investigation is all the more urgent.

The present work is an attempt to supply the missing data. In it are described the gametophytes, endosperm, embryo, and those parts of the sporophytic generation concerned with reproduction. In almost all cases the development of the structures concerned is described. Finally the bearing of the results obtained on the general problems such as those just outlined is discussed. The data have been obtained from several species of the genus. Two species representing the extremes of variation have been studied in practically all stages, and parts of the life history of several other species were determined.

2. History

The history of the work on the morphology of the inflorescences and flowers was narrated very clearly and completely in 1913 by Lignier and Tison (17) to whose article the reader is referred. The work on the gametophytic generation prior to 1899 was carefully reviewed by Lotsy (19). As this is the subject with which we are primarily concerned in the present work, the principal publications should be mentioned. They are those of Karsten (12, 13 and 14), of Strasburger (25) and of Bower (5). All these contributions and that of Lotsy give us only fragments of the life history. And since the publication of Lotsy's work in 1899 very few articles have appeared dealing with this phase of the life history. In a further contribution Lotsy (20) claimed that parthenogenesis may occur in *G. ula*. In 1908 Coulter (7) described a mature embryo-sac and some early stages in embryo-formation in *G. gnemon*. In 1912 Pearson (22) described some early stages in the male gametophyte of *G. africanum*.[1]

3. Materials and Collections

The material on which this investigation is based was collected during a visit to the Malay Archipelago in 1913. Most of the collecting was done in the Botanic Garden at Buitenzorg, Java, and in the adjacent country. Collections were also made at several other localities in Java and at Singapore.

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[1] Since the present paper went to the printer a more recent article by Pearson has appeared (Jour. Linn. Soc. **43**: 55–65, 1915) in which he described the strobili of Gnetum and the development of the endosperm.

In the famous Botanic Garden at Buitenzorg are excellent specimens of several species of the genus. From these was secured almost complete male material of *G. latifolium*, *G. moluccense*, *G. neglectum*, and *G. ula*, as well as of two unnamed species, *G. sp. 33*, and *G. sp. Borneo* of the Garden records.[1] Of female material almost complete stages of *G. moluccense*, *G. neglectum* and *G. sp. 59*, were obtained in the garden. Outside the garden one may find plenty of trees of *G. gnemon* in any native village where they are cultivated for the edible inflorescences and fruits. These trees, however, are almost all female. It appears that the natives destroy the male trees because they do not bear fruit, not knowing that pollination is necessary before fruit will be borne on the female trees. Consequently it is very difficult to secure male material or the fertilization and later stages of the female.[2] By prolonged search, however, I succeeded in finding in two villages (Tjidoerock and Tjipatat) a few male trees in close proximity to female specimens and from these it was easy to obtain the desired stages.

In the nearly impenetrable jungles at the base of Mt. Salak one can secure an abundance of material of *G. funiculare*. In isolated situations in the forest one can rarely find *G. neglectum* and *G. latifolium*.

With the exception of *G. gnemon* all these species are vines which are strikingly Dicotyledonous in appearance. The wild specimens prefer to climb among the branches of the tallest trees. The lower part of the stem is usually naked for a length of twenty to fifty feet, the leaves occurring only up among the branches of the tree to which the specimen is clinging. Some species have the strap-shaped stems of typical lianas. *G. gnemon* differs from the others in being an erect and often stout tree. It will appear later that this species is also very distinct from the others in its gametophytic generation.

There appears to be no definite flowering season in the case of *G. gnemon*. One may find all stages at almost any time of year.

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[1] The whole systematic classification of the genus is in urgent need of revision. I believe this is to be undertaken very soon by Dr. Valeton of the Buitenzorg Garden and consequently I have considered it advisable to retain the old names and numbers of the garden records, pending the publication of Dr. Valeton's study.

[2] It may also be that there is something peculiar in the sex determination of this species, for I have sometimes found groups of young specimens growing wild where they had apparently never been molested, and yet they were almost invariably female.

Of course the individual trees do not flower continuously but may begin to flower at any time. Between the appearance of the strobili and the formation of young green fruit a period of two or three months elapses. In the males as in so many tropical trees there are several flowering periods. One crop of inflorescences appears, matures and falls within a few weeks and within another few weeks another crop appears. The remaining species have more definite flowering seasons. Most of them begin about February in Java but the height of the season is reached in May. Individual specimens may not flower until long after this. Consequently in May and June one may obtain at the same time on different specimens many stages in the life history. When a specimen of *G. ula* begins to flower in February, young fruit with a large endosperm and suspensors will be present in May.

4. Inflorescences

1. *Normal.* (*a*) *Staminate.*—The staminate inflorescences usually appear in the axils of leaves, often in the old axils from which the leaves have fallen. As in the case of vegetative branches, it appears that new inflorescences may develop year after year in the same axil. Often two inflorescences will appear in the same axil at the same time. Frequently they are terminal. Frequently, too, they occur on the old wood of stout stems particularly on the naked parts of the stems of climbing species.

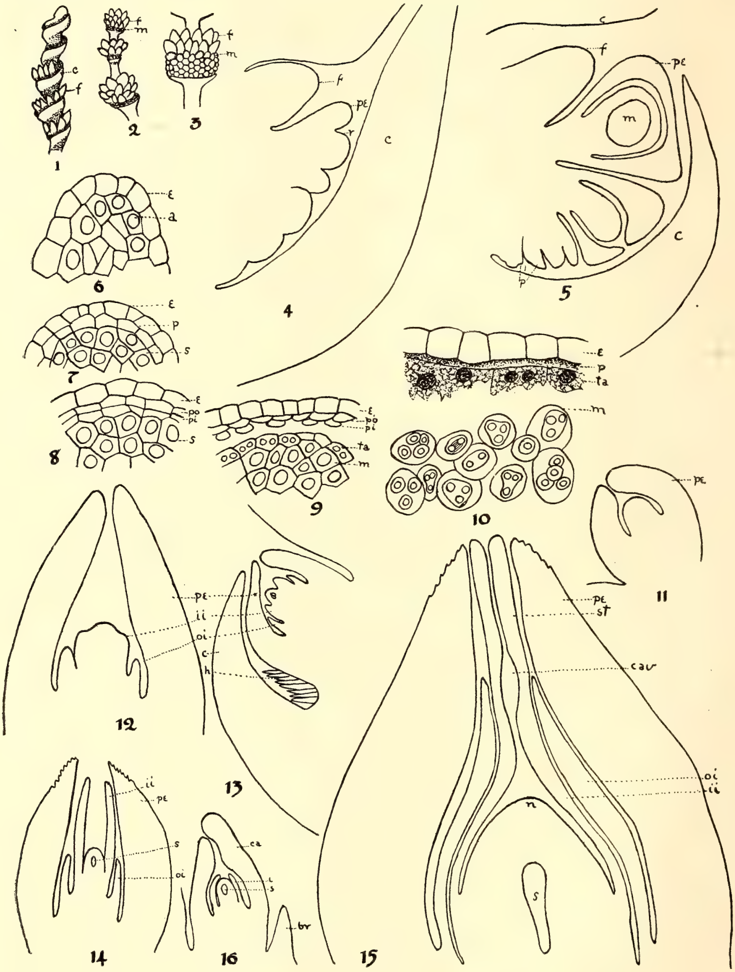
In some species each inflorescence consists of a single strobilus (*G. moluccense*). In others three strobili form a ternately divided inflorescence although the two lateral ones may be suppressed (*G. gnemon*). In others again the whole inflorescence may be profusely branched or paniculate (*G. sp. 33*). The strobilus itself, as is well known, normally consists of an axis bearing a series of collars (connate bracts) in which the flowers are borne attached to the axis. The constituent bracts of the lowermost collar are often distinct. The flowers are numerous and occur massed together in a low spiral above each collar. Invariably above each set of staminate flowers are abortive ovulate ones normally arranged in a single ring. The invariable occurrence of these abortive ovulate flowers in staminate strobili is a fact not sufficiently appreciated. Certain abnormal staminate strobili which appear to have considerable significance are described in connection with similar ovulate ones which are commoner and more easily interpreted.

(*b*) *Ovulate.*—The ovulate inflorescences, like the staminate, may be axillary, terminal or on old wood. Like the staminate ones, too, they may consist of a single strobilus or be more or less branched and paniculate. For any given species the particular form of the inflorescence is the same in the female as in the male. Each strobilus further resembles the male ones in consisting of a series of cups with flowers in their axils. But the cups are not contiguous as in the male and there is normally never more than a single cycle of flowers above each one. I have never found an ovulate strobilus bearing staminate flowers.

2. *Abnormal.*—Several kinds of abnormal inflorescences have been observed which will be described in full in a subsequent paper. Only two kinds need concern us at present. In one the bracts do not form a series of collars but a continuous spiral. In other words the flowers are arranged in a spiral and not in cycles, as is usual (see fig. 1, Plate II). The turns of the spiral are about the same distance apart as are the cycles of the normal. Some strobili are partly spiral and partly cyclic. Sometimes the spiral elevation corresponding to the collars is broken up into a series of bracts. These abnormal strobili may be either staminate or ovulate but more frequently they are ovulate. They are by no means rare. I have observed them in every species with which I have worked. On some specimens no other kind of strobili was found, indicating that it is perhaps an hereditary character.

Although the anatomy of these strobili has not yet been studied certain inferences seem to be justified. It seems clear that they represent a return to an ancestral condition. The spiral arrangement is the prevailing one in all groups of Gymnosperms except the Gnetales and these abnormal strobili merely represent reversions to that primitive Gymnosperm condition. The cyclic arrangement in the strobili of Gnetum has then been recently acquired.

It should be pointed out further that these strobili bear a remarkable resemblance to the catkins of the lower Dicotyledons. There is a central axis bearing bracts arranged in a spiral and in the axils of these bracts are borne the flowers just as in the Amentales. Furthermore, evidence will be presented later (page 18) to show that the female flowers of Gnetum have an ovary and perianth similar to those of the Amentalean flowers. It is therefore evident that these abnormal strobili of Gnetum are remarkably similar to those of the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lowest Angiosperms. Certainly if this type of Angiosperm was evolved from any forms at all closely related to the Gnetales it must have been while the latter bore strobili of this spiral type.

The second kind of abnormal strobilus is illustrated in figures 2 and 3. It is a staminate one with many abortive ovulate flowers massed in several ranks in place of the usual single ring. The strobilus represented in figure 2 is an old one from which the staminate flowers have fallen and on which the ovulate ones have grown considerably. Figure 3 shows the young condition. If one considers the male and female flowers as single stamens and ovules respectively, each provided with an envelope, the resemblance of each group with its collar to the Ranalean type of flower is striking. Above is a large group of ovules; below these, numerous stamens arranged in a low spiral; lower still the bracts of the collar. This is the arrangement in that type of Angiosperm flower which is considered by many botanists to be the primitive one. Nevertheless it seems clear that this resemblance is only superficial. Evidence will be presented later to show that both male and female flowers are themselves reduced from the bisporangiate condition.

3. *Anatomy.*—Only a preliminary study of the anatomy of the strobili, either normal or abnormal, has yet been made. But, in addition to the presence of centripetal wood, it has revealed the occurrence of a type of vessel not seen elsewhere in Gnetum. This is the familiar type of Ephedra. It will be recalled that the ordinary vessel of Gnetum has a single terminal perforation like the vessels of most Angiosperms. In fact the possession of this type of vessel is perhaps the most remarkable point of resemblance between Gnetum and Angiosperms. But in the axis of the strobilus, an admittedly conservative region, in place of the single large perforation the vessels have a series of enlarged bordered pits from which the middle lamellae and tori have disappeared. Now this is the ordinary type of vessel of Ephedra. Therefore in this conservative region there persists a type of vessel characteristic of the most primitive member of the Gnetales—a type which has evidently been derived from the Coniferous tracheid (Thompson, 27). The importance of these vessels in connection with the origin of the Angiosperm vessel is obvious. The difficulty arises, however, in that the primitive type of Angiosperm vessel appears to have scalariform end walls and not a single perforation.

5. Staminate Flower and Microsporangium

The young stamen is completely enclosed in an envelope known as the perianth which becomes ruptured at maturity. Stamen and envelope together constitute the staminate flower. The mature stamen resembles that of Angiosperms very closely except that it bears two sporangia instead of four. When it is remembered that the microsporophyll of no other group of Gymnosperms approaches this form, it seems that the resemblance to the Angiosperm stamen has not been sufficiently emphasized.

In the course of development the numerous flowers of a group arise in basipetal succession. Within a single collar one may find many stages. In the case represented in figure 4 the uppermost flower has already developed a perianth while the lowermost is a barely recognizable rudiment. In figure 5 the uppermost flower is in the mother-cell stage while the lowermost is just beginning to form a perianth. It is not possible to say that either perianth or stalk arises first because they arise together in a single rudiment from which the perianth becomes separated later by a circular depression (fig. 5 at the bottom). Then the perianth elongates and closes over the central rudiment of stalk and sporangia.

The archesporium consists of a hypodermal layer of cells which first becomes recognizable shortly after the perianth is differentiated (fig. 6). As usual it divides to form a primary parietal layer against the epidermis (fig. 7) and the primary sporogenous cells. The parietal layer divides again periclinally producing two layers of cells between the epidermis and tapetum (fig. 8). No further divisions take place in the wall cells. Indeed in some cases even the second layer is not formed and in other cases only a few of the primary wall cells appear to divide again. In all cases the cells of the inner layer become more or less rounded and separate from each other (fig. 9). Later the cells of the outer layer also become rounded at the ends. All the parietal cells then gradually degenerate leaving only a thin band of granular substance against the epidermis. Consequently there is nothing resembling the endothecium of Angiosperms. Indeed in the mature sporangium the spores are enclosed in a single layer of cells, the epidermis.

In the meantime the primary sporogenous cells have been dividing and have formed a considerable mass of tissue. Before the cells of the parietal layers begin to separate from each other a cleft appears between them and the sporogenous tissue. The outermost layer of sporogenous cells then quickly takes on the characters of a tapetum, both cytoplasm and nuclei becoming very dense and two nuclei appearing in each cell (figs. 9 and 10). In this case at least there can be no doubt that the tapetum is derived from sporogenous tissue. By the time the tapetum is fully differentiated the sporogenous cells within it have reached the mother-cell stage. The latter cells become more or less rounded and separated and then follow the tetrad divisions which produce the pollen grains. These divisions are not simultaneous throughout the sporangium as in Angiosperms but within a single sac all stages may be found from the undivided mother-cell to the young spore (fig. 10). Both tetrad divisions are completed before walls are formed. The four pollen grains occupy only a small part of the space within the mother-cell. Before the tetrad divisions are completed the granular substance referred to is all that remains of the parietal cells, and the tapetum has also begun to disintegrate (fig. 10.) When the young pollen grains become free the whole sac consists of a single layered epidermis, the granular remains of tapetum and wall cells and the mass of young microspores.

At maturity the stalk of the stamen elongates, breaking through the perianth and projecting beyond the collar of the strobilus. Dehiscence occurs by means of a cleft at the top of each sporangium. The first stamens to protrude are, of course, the uppermost ones. These soon fall and their places are taken by those next below and so on until all the stamens within the collar have matured.

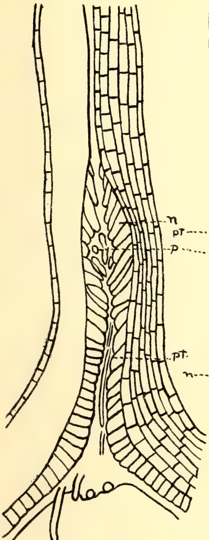
The period of time occupied by the events just described is much shorter than is usual in Gymnosperms. The whole course of development up to the shedding of the pollen takes place in a few weeks. The only Gymnosperm which approaches Gnetum in the rapidity of this development is Ephedra, another member of the Gnetales, in which according to Land (15) the staminate strobilus is first recognizable in December (in New Mexico) and the pollen is shed in April. Indeed the period of development is shorter than in even the spring-blooming Angiosperms in which as a rule the winter is passed in the mother-cell stage. The summer-blooming Angiosperms it seems are the only seed plants whose microsporangia develop so rapidly.

6. Ovulate Flower and Megasporangium

1. *Fertile.* (*a*) *Envelopes.*—The rudiment of the whole structure is first recognizable as an elevation just above a collar of the strobilus. From the periphery of this elevation a thick, many-layered envelope is differentiated which grows up and encloses the central protuberance (fig. 11). This envelope becomes the so-called perianth. After it has enclosed the central rudiment another and thinner envelope is differentiated in similar fashion. While the latter is still very small the depression appears which separates the third and innermost envelope from the nucellus (see fig. 12, in which this circular depression is barely recognizable). The three envelopes thus develop in acropetal succession. In all the species investigated by the writer this is the order of development. This statement is in accordance with the observations of the earlier investigators (Strasburger, 25) and contrary to those of Coulter (7) who states that the inner envelope appears before the middle one.

The inner envelope soon elongates enormously (figs. 13 and 14). It quickly extends beyond the middle envelope and later even beyond the outer one to form the so-called style. At pollination time its tip becomes flaring and lacerated and always holds a droplet of liquid in which the pollen grains lodge. Its inner structure undergoes a significant development. All the cells except the innermost layer remain small and elongated parallel to the axis of the style. The cells of the layer lining the cavity, however, become large and elongated at right angles to the axis (fig. 17). Their rounded ends project into the cavity. Their protoplasm and nuclei become very dense and deeply staining (fig. 18). In fact the whole layer appears to be nutritive. A little below the middle a considerable chamber is formed in which many of the pollen grains lodge, appearing to stick first against the projecting ends of the nutritive cells. Here the pollen grains germinate and the tubes grow down to the nucellus. The nutritive layer becomes disintegrated into a granular mass by the growth of the tubes. After the pollen grains germinate the passage above the chamber closes.

Not all the pollen grains germinate in the style. Indeed most of them germinate on the tip of the nucellus (fig. 35). Nevertheless the style is much more like that of Angiosperms than had been supposed. It not only catches the microspores but also serves to conduct



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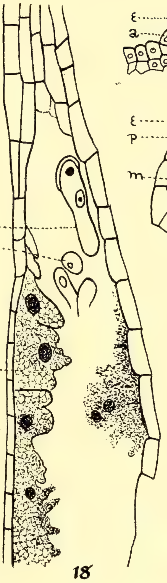

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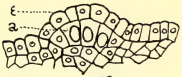

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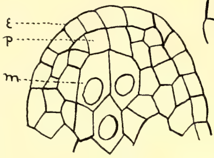

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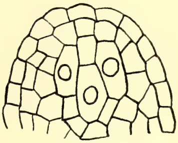

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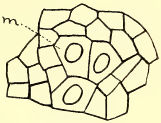

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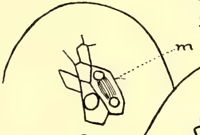

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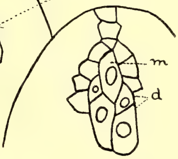

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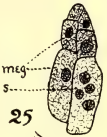

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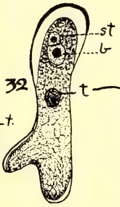

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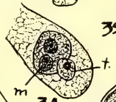

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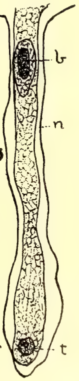

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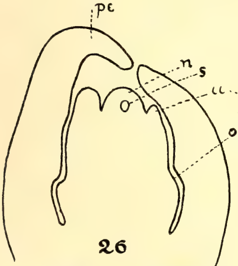

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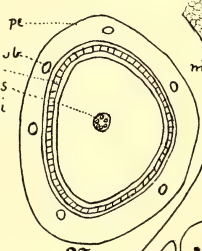

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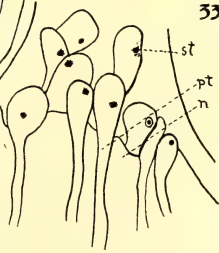

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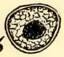

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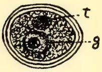

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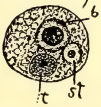

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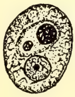

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and nourish some of the pollen tubes, and has a particular tissue developed for this purpose. This is a fact which must be given much weight in regard to the morphology of the envelopes of the female flower. At first sight it offers strong support to the contention that the inner envelope is really an ovary homologous to that of Angiosperms.

Moreover the fact that the pollen grains may germinate at a distance from the nucellus is a point which in itself closely connects Gnetum with the Angiosperms and removes it from the Gymnosperms. As stated by Coulter and Chamberlain (8) "the chief contrast in the sporophyte is that in Gymnosperms pollination results in bringing the pollen grains in contact with the ovule while in Angiosperms the result of pollination places the pollen in contact with a receptive surface developed by the carpel." Whatever be the morphology of the inner envelope the essential point is that some of the pollen grains do not germinate on the nucellus as in Gymnosperms but at a distance from it as in Angiosperms. And by many botanists this is considered the chief contrast between the sporophyte of Gymnosperms and that of Angiosperms.

That part of the inner integument surrounding the nucellus remains thin and undifferentiated. At the maturity of the seed it forms a thin, more or less papery covering of the endosperm. The middle envelope becomes differentiated into two tissues, an inner hard, stony layer, and an outer thin papery one, closely investing the stony layer and containing many sharp spicular cells. This outer layer of the middle envelope is quite thick at the top of the endosperm. The outer envelope becomes very thick. Its tip is papillate. It contains many resin passages and star-shaped spicular cells. At the maturity of the seed it forms a thick, fleshy, edible layer of a bright red color.

Concerning the morphology of these envelopes there have been many opinions which may be summarized as follows: (1) All three are integuments resulting from the differentiation of the single integument of Gymnosperms (Strasburger, 25); (2) the two innermost ones are integuments and the outermost is a perianth (Beccari, 3) or something analogous to it (Coulter, 7); (3) the two innermost ones are integuments and the outermost is an ovary or something analogous to it (van Tieghem, 32); (4) the innermost one is a true Angiospermous ovary and the outer two perianth or something analogous to it (Lignier and Tison, 17 and 18). These views seem to include all the possibilities and it will perhaps be difficult to choose between them until the anatomy is studied in a wider range of forms. Nevertheless the conditions described in the preceding pages have a bearing on the problem which should be pointed out.

In regard to the view that all three envelopes are integuments it is only necessary to remark that there is no evidence except their general appearance in favor of it. Such a view, moreover, fails to offer any explanation of the envelope of the male flower which is evidently of the same type.

The second view, namely that the two inner envelopes are true integuments and the outer a perianth or something analogous to it is the one most generally held at the present time and most convincingly stated by Coulter (7). This author pointed out that those coverings of the ripe seed which are derived from the two inner envelopes are the same as those derived from the single integument of Gymnosperms: an inner fleshy, a middle stony, and an outer fleshy. The inner fleshy layer of Gnetum is derived from the inner envelope and the other two from the middle envelope. Accordingly Coulter concluded that the inner envelope of Gnetum represents the inner part of the single integument of Gymnosperms and the middle envelope represents the remainder of this single integument. In other words the single integument of other Gymnosperms has become divided into two distinct integuments in Gnetum. Opposed to this view is the style-like character of the projecting portion of the inner envelope which strongly supports the view that it is really an ovary and not an integument. Further, the development and anatomy of this envelope in both Ephedra and Welwitschia indicate that it really consists of two fused members.

The third view (that there are two integuments and an ovary) is at first sight very attractive particularly when this flower is compared with that of one of the lower Angiosperms such as Peperomia. Figure 16 represents a section of a flower of Peperomia *sp.*, and if it is compared with a section of a Gnetum flower it is seen that the resemblance is very striking and that the carpel of Peperomia corresponds closely to the outer envelope of Gnetum. Moreover the development of the flower of Peperomia is almost a repetition of that of Gnetum (Johnson, 11). In form and position with respect to the remainder of the flower this envelope certainly resembles an ovary as much as a true perianth. The objections to this view are as follows: (1) As previously shown the anatomy and development of the inner envelopes of other members of the Gnetales indicates that they are composed of two fused foliar members and that they are not integuments. (2) The outer envelope has not the functions of a carpel in collecting pollen and conducting pollen tubes. (3) The envelope of the staminate flower of Gnetum is obviously of the same type and certainly cannot be considered an ovary. The homology of these envelopes in male and female flowers is shown by their form, development and anatomy. (4) The similar envelope of the male flower of Welwitschia encloses the stamen cycle and therefore cannot be considered an ovary. These objections seem to constitute too great a body of evidence for the view to be longer tenable, despite the evident similarity in form between the outer envelope and a true ovary.

Finally there is the theory recently advanced by Lignier and Tison (17, 18) that the innermost envelope is a true ovary and that the two outer envelopes are in the nature of a perianth. This implies that the ovule is destitute of integuments. There appears to be much more evidence in favor of this view than any of the others partly because of the facts revealed in this article and partly because it accounts for the morphology of the similar envelopes of other Gnetalean flowers and indeed for their whole structure. It is clear that in any theory of the morphology of the envelopes in Gnetum, the structure of the female flower of Gnetum must be harmonized not only with that of the male flower but also with that of the flowers of the other genera of the Gnetales. The general argument as developed by Lignier and Tison, chiefly in connection with the flower of Welwitschia, will first be stated and then applied to Gnetum.

It is admitted by practically all investigators that the structure of the male flower of Welwitschia with its abortive ovule above the cycle of stamens indicates that the immediate ancestors of the genus bore hermaphrodite flowers arranged on the Angiosperm plan, and that the female flower has resulted from the suppression of the stamen cycle. Owing to the similarity of the flowers of other members of the Gnetales to those of Welwitschia it is evident that they too are reduced from a hermaphrodite condition. This conclusion is independently confirmed by the discovery (18) of abnormal flowers of *Gnetum scandens* bearing stamens within the second envelope. With this general arrangement in mind we may now consider the morphology of the individual parts.

That which is called the integument of the Welwitschia ovule is, according to the theory, really an ovary. Lignier and Tison justly lay much emphasis on the style-like character of its projecting tip. Furthermore we have already called attention to the fact that its development and anatomy both in Ephedra and Welwitschia indicate that it is an ovary of two fused members. The stamen cycle of Welwitschia is fused at the base and divided above into six parts. But its anatomy shows that there are really two members (as in the other cycles) which branch above. This is assumed to be the ancestral condition for the group. The perianth consists of two pairs of bracts. In Welwitschia the first pair are connately used and the second pair represented by bractlets. In Ephedra both pairs are connate. The ancestral flower of the Gnetales therefore consisted of an ovary of two fused members, two stamens and two pairs of decussate bracts.

It must be admitted that one finds difficulty in imagining how the staminate flower of Gnetum can have been reduced from such a type. This flower consists of a single stamen surrounded by a single envelope. It must be assumed that the ovary, one stamen, and one pair of perianth bracts have disappeared and that the other stamen has taken a position at the top of the axis. Although this seems a big assumption to make I believe it is justified particularly in view of the certain abnormalities which will be described in full elsewhere. They consist in brief of male flowers which had grown out into axes of considerable length and complexity.

We are now in a position to apply the general conception of the Gnetalean flower to the structures in the female flower of Gnetum. It is plain that the inner envelope of Gnetum is even more like an ovary than that of Welwitschia for it not only resembles an ovary in form and anatomy but it also bears a style on which the pollen is caught and in which some of it germinates. Accordingly we conclude that the first envelope is really an ovary whether or not it is the homologue of the Angiosperm ovary. The second and third envelopes then represent fused bracts which may or may not constitute a true perianth. The stamen cycle has disappeared. The whole view receives strong support from the discovery of abnormal flowers bearing stamens within the second envelope.

This body of evidence seems to demonstrate that we have at last obtained the proper interpretation of the envelopes of Gnetum.

Not only does it satisfy all the conditions in Gnetum but it also harmonizes these conditions with those in both the other genera of Gnetales.

The conclusions which have just been reached have a profound significance in connection with the relationship between Angiosperms and Gnetales. If the reduction hypothesis is correct the ancestral flower of the Gnetales had all the parts of the Angiosperm type arranged in the same manner as that type. We may now enquire whether those individual parts correspond exactly.

It is clear that what we have called an ovary in Gnetum is in all essential respects the same as that of Angiosperms. It is a sac derived from foliar members enclosing an ovule and bearing a special structure on which the pollen is received and in which some of it germinates. The real question appears to be whether Gnetum is a true Angiosperm. For all practical purposes it is Angiospermous.

The ovule is single and orthotropous, rising from the base of the ovarian cavity. These are the conditions in some of those Angiosperms which are classified on the basis of other characters at the bottom of the phylum—the Amentales. Some of the latter, *e. g.*, Salicaceae, have more than one ovule in each ovary but even in Gnetum I have seen in abnormal instances two ovules developing in an ovary. The chief difference as far as the ovule is concerned is the absence of integuments in Gnetum (according to our interpretation). But the ovules of certain Angiosperms also lack integuments and it is not strange that the integuments should disappear in flowers reduced in so many other ways as are those of Gnetum.

In most Angiosperms the ovules are attached to the side of the sporangial cavity and are therefore said to be foliar. Those which rise from the bottom of the cavity are obviously attached to an axis and are said to be cauline. The old view that ovules are modifications of some part of a leaf implied that the foliar condition is primitive and that the cauline one has been derived from it. This conclusion is opposed by the evidence derived from the distribution of the two types, the cauline condition being found chiefly among primitive forms and the foliar among the higher families. The mistake lies in the original assumption that ovules are modifications of some part of a leaf. Ovules are ancient members of the plant body and hold no necessary relation to either leaf or stem. As stated by Coulter and Chamberlain (8) they have a history which probably antedates that of both leaf and stem. There is no reason why they cannot occur on the base as well as on the sides of the sporangial cavity. Therefore the obviously cauline character of the ovules of Gnetum cannot be used as an argument against its being related to the type from which Angiosperms were derived. Indeed it rather supports that view because cauline ovules are found chiefly among primitive Angiosperms.

The stamen of Gnetum has already been compared with that of Angiosperms.

The perianth of the Gnetales according to our interpretation consists of two pairs of connate bracts. Among the Amentales the perianth consists of similar small colorless bracts which are either distinct (*e. g.*, Myricaceae), or fused (*e. g.*, Juglandaceae, Betulaceae). Therefore it appears that there is more than mere analogy between the perianth of primitive Angiosperms and that which we have called perianth in Gnetum.

We conclude that in regard to every part the flower of Gnetum closely resembles that of primitive Angiosperms. And we have already seen that the arrangement of the flowers themselves particularly in the abnormal spiral strobili is just the same as in those primitive Angiosperms. The flower of Gnetum is, therefore, not far removed from the type from which that of Angiosperms was derived.

This view implies that the Amentalean flower is the most primitive type found in Angiosperms. Another admittedly primitive type is that of the Ranales with numerous carpels, stamens and floral leaves all arranged in spirals. But if the Gnetalean derivation of the Angiosperms is the correct one this type must be considered more specialized than the Amentalean one. In this connection it is interesting to recall the structure of those abnormal strobili previously referred to in which there is a mass of ovulate flowers above the usual set of staminate ones. If the Gnetalean flowers be considered as simple ovaries and stamens and not as reduced from a hermaphrodite condition, the resemblance of these abnormal strobili to the Ranalean flower is marked. But all the evidence indicates that they are really reduced and therefore we must conclude that this resemblance is only superficial.

(*b*) *Nucellus and Archesporium.*—As stated by Strasburger (25) the archesporium always consists of two or more hypodermal cells. I have examined an abundance of material in all the early stages and have always found that as soon as an archesporium is recognizable it consists of at least two cells and usually of three or four (fig. 19). It makes its appearance at about the time that the inner envelope (ovary) becomes differentiated. The succeeding events take place in the usual manner. The archesporial cells by periclinal walls cut off the primary wall cells (fig. 20) which, together with the epidermis develop a large mass of tissue above the sporogenous cells. The cells of the upper part of the nucellus become densely charged with cytoplasm which contains many starch grains. No definite pollen chamber is formed though the tip of the nucellus usually becomes disorganized by the ingrowing pollen tubes. That part of the nucellus at the base of the embryo-sac develops a peculiar nutritive pavement tissue which according to Coulter (7) was mistaken by Lotsy (19) for tissue within the sac. But it will appear later that Lotsy was correct in stating that there may be cellular tissue in the base of the sac when there are only free nuclei at the top. It will also appear, however, that Lotsy was mistaken in stating that this cellular tissue was present before the pollen tube enters the sac. It is in reality endosperm tissue which develops after the entrance of the pollen tube and is quite distinct from the pavement tissue outside the sac.

The whole ovule undergoes a rapid development while the pollen tubes are growing through the nucellus. Soon after pollination time (indicated by the flaring style with its droplet of liquid) certain ovules are seen to be growing rapidly while others remain the same size. One naturally concludes that the growing ovules have been fertilized and that the endosperm is forming. On sectioning such ovules, however, one invariably finds that the pollen tubes have not yet reached the sac. Not until the ovules are considerably larger than those which failed to be pollinated does one find pollen tubes in contact with the embryo-sac. It seems, therefore, that the presence of pollen tubes in the nucellus stimulates the latter to further development.

(*c*) *Sporogenous Tissue.*—According to Strasburger (25) the primary sporogenous cells divide to form the mother cells, a very unusual behavior in Gymnosperms. My sections show clearly that this is not the case but that the primary sporogenous cells as usual function directly as mother cells. There are accordingly from two to four of the latter. Figures 20 and 21 show these cells in longitudinal section and figure 22 in transverse section. The mother-cells divide to form the megaspores while the ovule is still very young—in fact while there are only three or four rows of cells between the sporogenous tissue and the epidermis. Figure 23 represents a case in which one mother cell is dividing while the one beside it remains undivided. In figure 24 the two deeper lying cells have divided once and the more superficial cell has remained undivided. In figure 25 are represented the products of two mother cells. At the left is a linear row of three cells showing that one daughter cell only has divided to form megaspores. At the right are three similar cells the lowermost of which (megaspore) has divided and produced a four nucleated embryo-sac. In general the outer daughter cell of the first division fails to divide again. So far as I have observed it is always the deepest megaspore which functions. Invariably two and frequently three mother cells produce megaspores. In case one does not divide it is always the outermost. Usually a megaspore from each mother cell which divides develops into an embryo-sac. For this reason there are almost always more than one embryo-sac in a mature ovule, usually a large central one and one or two smaller ones at its outer end.

2. *Abortive Megasporangia.*—It will be recalled that in every staminate strobilus there is a ring of abortive ovulate flowers above each set of staminate flowers. It has been reported (Lotsy, 19) that these flowers occasionally produce fruit and this statement I can confirm. It is my observation that while the great majority of staminate trees never produce fruit from these ovules an occasional tree will produce large numbers. If one finds fruit developing in a staminate strobilus one is almost certain to find many other examples on the same tree. It seems therefore to be a definite tendency, probably inherited, in certain trees and not an occasional event on any tree. Of course the axis of the strobilus becomes much stronger and thicker than it otherwise would. Frequently the axis is unable to develop strength enough to nourish these abnormal fruits for often I have seen staminate strobili with several half developed fruits becoming yellow and sickly. Though very few of the ovules in staminate strobili produce fruit a good many of them develop embryo-sacs which appear to be of the usual type. Figure 27 shows a transverse section of an abortive ovule with the embryo-sac in the free nucleate condition.

It is always stated that these flowers differ from the typical flowers of the ovulate inflorescence in that they have only two envelopes.

The relationships of these envelopes to those of the typical flower were not understood. I have observed that every abortive ovulate flower at a certain stage in its development possesses a rudiment of a third envelope between the two well-developed ones (see fig. 26). It seems evident, therefore, that the envelopes present are the inner one (ovary) and the outer which we call perianth and that the middle envelope is represented only by the vestige present during development. The invariable presence of the middle envelope although in a rudimentary condition, together with the frequent presence of a typical embryo-sac and the rare development of fruit, shows that these flowers are quite homologous with the functional ovulate flowers. And this homology suggests that the immediate ancestors of Gnetum had bisporangiate strobili. In view of the conclusion that the flowers themselves were originally bisporangiate (page 18) and have been reduced to the monosporangiate condition, it is difficult to understand why the bisporangiate strobili should have been developed and then reduced.

7. Male Gametophyte

Until the publication of Pearson's (22) incomplete account of conditions in *G. africanum* our only knowledge of the male gametophyte of the genus was the statement of Lotsy (19) that the tube nucleus and two male cells of the ordinary type are in the pollen tube just before fertilization. The writer has been able to observe almost all stages in the development of the male gametophyte in *G. gnemon*, *G. latifolium* and *G. sp. 33* and several stages in other species. The different species are quite alike in all essential points.

The young microspore on being freed from the cavity of the mother cell has a very thin wall in which only one layer can be distinguished. Later a second layer appears and the typical exine and intine are present. The exine becomes covered with very small protuberances.

The nucleus of the young microspore is large and rather dense and has a prominent nucleolus. This nucleus divides very soon after the microspore is freed. The first division does not give rise to a prothallial cell as in so many Gymnosperms but to a tube nucleus and generative cell (see fig. 29). No prothallial cells are formed. In this important respect, therefore, Gnetum has completely departed from the Gymnosperm condition and has arrived at the Angiosperm condition. The tube nucleus is slightly larger than the generative nucleus but is much less dense and has a very distinct nucleolus. In most preparations it is impossible to distinguish the cytoplasm of the generative cell from the general cytoplasm of the spore. But in well-stained sections the generative cytoplasm is seen as a narrow lighter band surrounding the generative nucleus, the whole cell being only slightly larger than the tube nucleus. The generative nucleus is very dense and deeply staining. Very soon the generative cell divides to form a small stalk cell and a body cell (fig. 30). In this condition the pollen grain is shed, and consequently at pollination time the gametophyte consists of a tube nucleus in the general cytoplasm, a body cell and a stalk cell. The tube nucleus is distinguishable by its greater size, lighter appearance and conspicuous nucleolus. It is very often difficult to see that the body nucleus and stalk nucleus are each surrounded by its own cytoplasm. The body nucleus is smaller than the tube nucleus and very dense. The stalk nucleus is very small and dense. Frequently the stalk and body nuclei appear to be in the same cytoplasm (fig. 31).

It is particularly easy to watch the growth of the pollen tube because as previously stated the pollen grains frequently germinate in the style. The exine is thrown off as usual and the cavity of the style in later stages frequently contains many of these outer coats. The intine grows out into a tube at the point nearest the tube nucleus. Figure 32 represents a tube which has branched though far from the nucellus. The tube nucleus with its conspicuous nucleolus is next to the branched end. Back in the pollen grain proper are the body and stalk cells, each with its own cytoplasm. As the tube grows the tube nucleus and body cell pass out into it (fig. 33). The stalk nucleus seems invariably to stay behind in the old grain. There it remains for a long time until finally it degenerates. Figure 35 shows the tip of a nucellus with many pollen grains which have germinated and sent tubes far down towards the embryo-sac. In all cases the tube nucleus and body cell have passed down the tube but the stalk nucleus has remained in the old pollen grain. Some of them have begun to disintegrate. Old germinated pollen grains, whether in the style or on the nucellus usually show remains of this stalk nucleus. So far as I am aware this behavior occurs in no other Gymnosperm. It seems to be a step towards the complete elimination of the stalk cell.

The body cell never divides until it is in the pollen tube. Figure

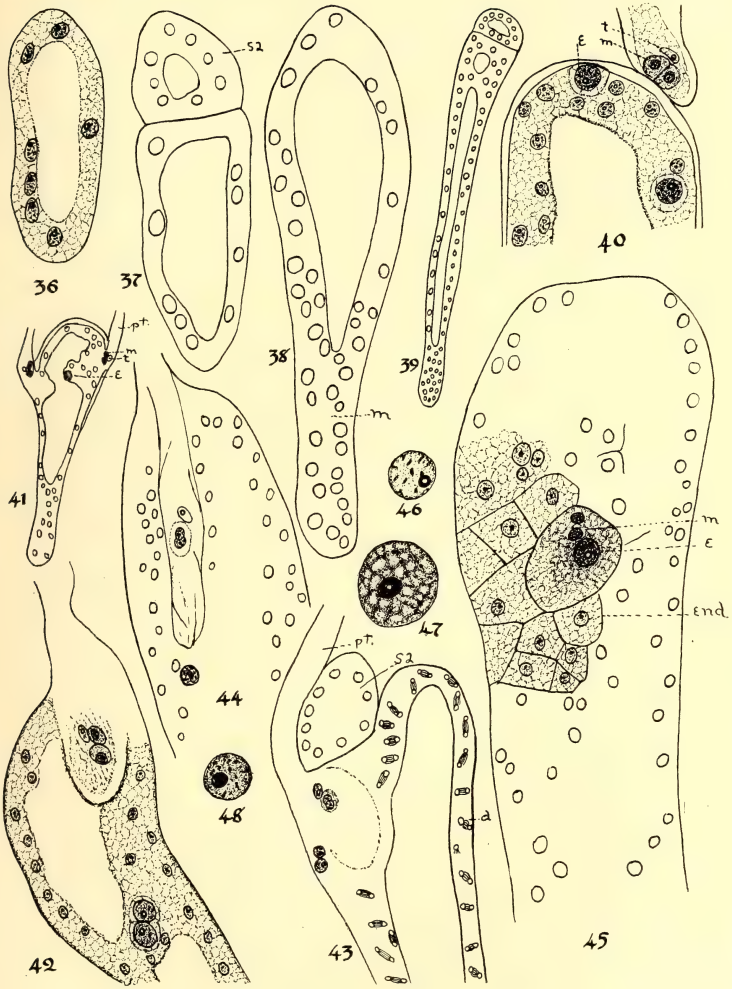
33 shows a tube in which the cell is just entering the nucellus. The tube nucleus is down at the tip of the tube. It will be observed that the body cell is undivided. Unfortunately I have never seen its division, but by the time it is half way through the nucellus it always contains two nuclei, consequently the division must occur in the upper part of the nucellus. It is usually impossible to see in my preparations that there are two distinct male cells. Generally I can see only two nuclei lying in a common cytoplasm (fig. 34). In favorable sections, however, one can see that this cytoplasm is divided into two by a delicate membrane (fig. 40). It may be that in all cases there are two distinct male cells but this I am not prepared to say. In the lower part of the nucellus the tube nucleus and male cells lie side by side, with the former in advance. Against the sac the tube nucleus is found at the side of or behind the male cells. The tube nucleus is always larger than the other nuclei and has a prominent nucleolus. There is no difference in size between the male nuclei or cells.

The most notable points in this gametophyte are (1) the absence of prothallial cells, (2) the germination of the pollen grains in the style as well as on the nucellus (3) the retention of the stalk cell in the pollen grain and (4) the division of the body cell in the nucellus. The first three points bring the male gametophyte of *Gnetum* very close to that of Angiosperms.

8. Female Gametophyte

As previously stated two or three megaspores usually develop into embryo-sacs. Each of these functioning megaspores is descended from a different mother cell. Figure 25 shows a four-nucleate embryo-sac and alongside it a megaspore derived from another mother cell. At the end of the sac and of the undivided megaspore are the sister megaspores which will not function.

The subsequent divisions in the gametophyte take place in the usual Gymnospermic fashion. A vacuole soon appears in the center of the developing sac and the protoplasm and nuclei become confined to a parietal layer. Figures 36 and 37 illustrate the conditions found in the young sac, the latter figure also showing the typical appearance of two sacs in one nucellus at this stage. Very often the vacuole appears first at the upper end of the sac (fig. 38) and gradually extends downward. In any case the upper end always becomes larger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than the lower, the whole then taking on the form of an inverted flask. Usually the vacuole does not extend completely to the bottom of the sac, this end being occupied by a mass of protoplasm and nuclei (figs. 38, 39). This fact is important in connection with the formation of the endosperm. Figure 41 shows the appearance of the mature sac in *G. gnemon* and figure 39 that of *G. sp. 33*. Each is seen to be shaped like an inverted flask with a mass of protoplasm and nuclei at the lower end and a thin layer of protoplasm containing a single row of nuclei along the sides. Occasionally strands of protoplasm containing nuclei stretch across the upper end. In *Gnetum gnemon* the neck of the flask is not nearly so long as in *G. sp. 33* and other species.

The nuclei have almost the same appearance throughout the development of the sac, but gradually get slightly larger. Each one contains very little chromatin matter and a large conspicuous nucleolus in the form of a hollow ball. The cavity at the center of the nucleolus is usually very plain. The divisions in the sac are simultaneous. The number of nuclei finally produced in *G. gnemon* is approximately 256, which is the usual number found in Gymnosperms before wall formation takes place. It is evident therefore that in this species the development of the gametophyte follows the typical Gymnosperm method in all stages prior to the formation of cells. At this point the similarity ends. In *G. sp. 33* and *G. moluccense* the total number of nuclei is approximately double the usual Gymnospermic number or 512. In other words another division of each nucleus has taken place.

As stated by Thomson (30) the megaspore membrane (wall of the embryo-sac) is very thin. In all the species examined I could distinguish only a single thin homogeneous membrane which almost disappears towards the top of the sac. In *G. sp. 33* this membrane becomes considerably thicker during the early stages of endosperm formation.

After the pollen tube comes in contact with the embryo-sac a very important development takes place. One or more of the nuclei at the upper end of the sac become differentiated from the others and definitely recognizable as egg nuclei. They can easily be distinguished from the remaining nuclei by their larger size, greater affinity for stains, very dense structure, and inconspicuous nucleolus. The other gametophytic nuclei always have a loose structure and very conspicuous nucleolus. It is not always possible to satisfy oneself that the egg nucleus has its own cytoplasm and limiting membrane.

I believe that the egg cytoplasm and membrane are always differentiated and it may be that in cases where they cannot be distinguished the egg nuclei have just been organized and that the cytoplasm and membrane will be differentiated later. In any case the nuclei themselves are always plainly differentiated. Usually two such eggs are present, often only one, and sometimes three. Figure 40 shows the upper end of a sac in which two eggs have been differentiated. The pollen tube is seen pressed against the sac. Figure 41 gives the appearance of the whole sac at this time. The eggs are also shown in the sacs in figures 42, 43 and 44. Figure 47 represents an egg nucleus alone.

Apparently these eggs make their appearance only under the stimulus of the presence of the pollen tube against the sac. It seems that the pollen tube remains pressed against the sac for a long time before bursting in, because it is found in this position more commonly than in any other in ovules of about this age. One can frequently find sacs in contact with pollen tubes although no eggs have yet been differentiated. But before the pollen tube enters the sac, the eggs are always visible. Therefore one concludes that the eggs become differentiated only when the pollen tubes are in contact with the sac.

The position of the nuclei which become transformed into eggs bears no definite relation to that of the pollen tube. Though they are always in the upper part of the sac they may be either directly under the pollen tube or at the opposite side of the sac and are often at some distance from the end.

The fact that Gnetum forms definite eggs is one of the outstanding results of this investigation. It has always been supposed that any of the free nuclei in the gametophyte might be fertilized (Lotsy, 19). "It is in this stage that fertilization occurs, for the free nuclei are potential egg nuclei, although a group at the antipodal end of the sac may be as distinctly vegetative as are the antipodal cells of Angiosperms" (Coulter and Chamberlain 8). If this is true then of course all the nuclei in an Angiosperm gametophyte are in the same sense potential egg nuclei. In fact the differentiation of special nuclei to serve as eggs makes the resemblance of the female gametophyte of Gnetum to that of Angiosperms still more striking. Particularly does it strengthen the resemblance, already frequently pointed out, to the irregular sacs such as those of Peperomia (Campbell, 6, Johnson, 10, 11), Gunnera (Schnegg, 23), etc., in which many free nuclei are found and in which no polarity is evident. Many such sacs have been described in recent years and it is by no means proven that they are specialized and not primitive as was originally contended by Campbell but disputed by Johnson. When the development of the endosperm is described (page 34) it will be seen that there is a still further resemblance. It seems particularly significant that Peperomia should also resemble Gnetum in many points of flower structure and that it should be classified by universal consent among the very lowest of the Angiosperms.

Whether or not the female gametophyte of Gnetum represents the condition from which the Angiosperm gametophyte was really derived, it seems to have a bearing on the morphological nature of the cells within the Angiosperm embryo-sac. Many attempts have been made to relate the egg and synergids to the archegonium of lower plants. It has been urged at different times (1) that they represent the egg and canal cells of a single archegonium, (2) that all three represent archegonia, and (3) that only the egg represents an archegonium while the synergids represent the upper part of the prothallus (Berridge and Sanday 4). The conditions in Gnetum show that there is no need to relate the eggs in any way to archegonia but merely to consider them as eggs produced by a gametophyte which cannot form archegonia. The absence of cellular tissue prevents the formation of archegonia and hence free nuclei organize as eggs. If this is true for Gnetum it is still more evidently true for Angiosperms even though there be no genetic connection between them.

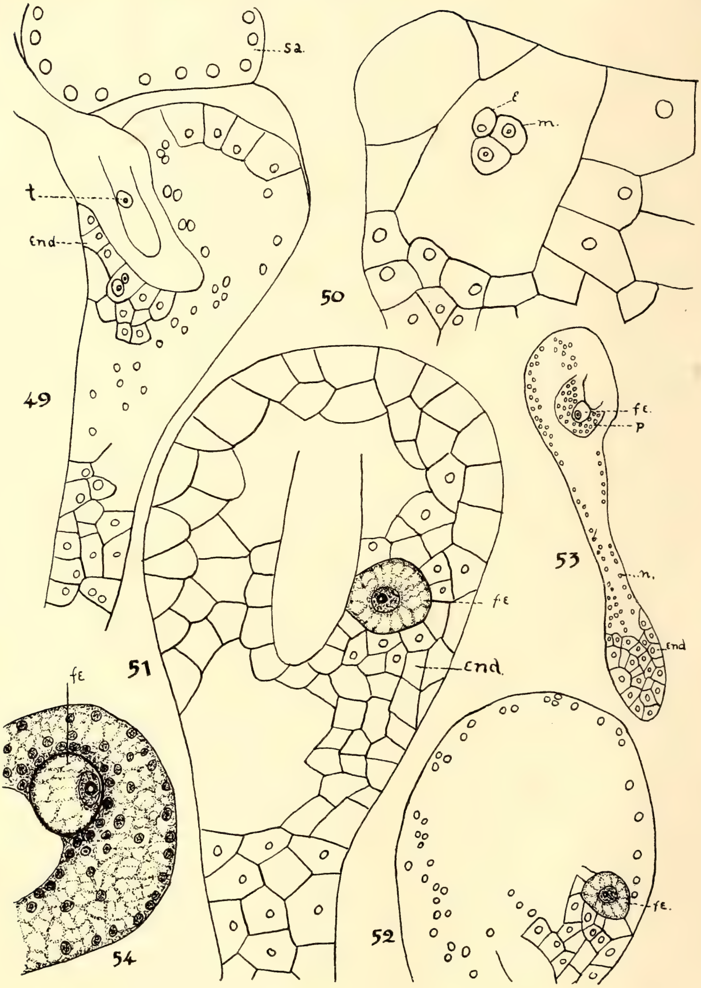
The conditions in the female gametophyte may be summarized as follows: the whole sac is shaped like an inverted flask; at the mouth of the flask is a considerable mass of cytoplasm and free nuclei; along the sides of the neck a narrow band of cytoplasm with one row of nuclei; in the body a broader band of cytoplasm with one or more rows of nuclei; in variable positions one or more eggs. The inferences to be drawn from these conditions are: (1) The early development is Gymnospermic, (2) the later development and mature condition is even more suggestive of Angiosperms, particularly of the irregular forms, than had been supposed, (3) it is impossible to relate any structures in this or the Angiosperm gametophyte with the archegonia of lower forms.

9. Fertilization

The entrance of the pollen tube into the embryo-sac will be described under the head of fertilization, although in some species at least several important events intervene between the entrance of the tube and the essential act of fertilization, the fusion of male and female nuclei.

The point of contact between pollen tube and embryo-sac varies in position but it is usually at the side of the expanded part of the sac. The tube nucleus takes up a position at the side of or behind the male cells. The contents of the pollen tube are discharged into the sac in the usual way. Both male cells, the tube nucleus, and a certain amount of cytoplasm pass in. The contents of the sac draw back slightly though not pushed back by any wall or mass of protoplasm. The male cells then make their way to one of the eggs, often traversing a considerable space in doing so, and leaving the tube nucleus behind. Figure 41 shows the general arrangement of the sac and tubes. One tube is entering at the left and another further up at the right. The egg is visible at the inner edge of a mass of cytoplasm. Figure 42 shows part of a sac under greater magnification. The male cells and tube nucleus are just entering and the two eggs are to be seen further down in the sac. Figure 43, introduced for another purpose, represents about the same stage. In the case represented in figure 44 the male cells have penetrated far into the sac towards the egg. The clear space ahead bounded by a partly collapsed membrane is typical. The subsequent events depend upon the species.

In *G. sp. 33* a considerable time elapses before the actual fusion of the nuclei, and in the meantime important events take place in endosperm formation. The egg and male nuclei are then found in a definite chamber partly or wholly surrounded by cells of the endosperm formed in a way to be described later (page 34). Figure 45 shows a large cell containing the egg nucleus and the two male nuclei, the whole cell surrounded by the cells of the endosperm. Further away are free nuclei. In figure 49 the cells are seen to have formed around the end of the pollen tube which contains the tube nucleus. A large cell in the center contains the egg and male nucleus. Down in the neck of the flask are the endosperm cells which extend right to the bottom of the flask. Figure 50 shows the two distinct male cells in contact with the egg, all three enclosed in a definite chamber.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The conspicuous fusion nucleus is relatively very large and is situated in the center of a large cell. It is quite impossible to distinguish the male and female chromosomes. In view of the independence of the maternal and paternal chromosomes until after the first division in many recently described cases, I have examined this phase of the life history very carefully but have always found that the two nuclear masses become quite indistinguishable and that later a single large nucleolus is formed. The cell containing the fusion nucleus is usually in contact with the old pollen tube. Sometimes the embryo-sac in which it is found is nearly filled with cellular endosperm; sometimes it contains many free nuclei.

In *G. gnemon* the sexual cells are not surrounded by a cellular endosperm. The cell containing them is, however, surrounded by a more or less definite mass of cytoplasm with many free nuclei. It is usually found either against the end of the pollen tube or the wall of the embryo-sac. I have not seen the actual fusion of sexual nuclei in *G. gnemon* and consequently cannot state exactly when it takes place with respect to the formation of this protoplasmic mass. Figure 53 represents the whole sac at this time and figure 54 the fertilized egg surrounded by the body of protoplasm and nuclei.

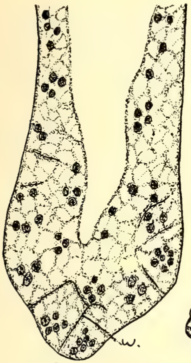
The possibility of the occurrence of typical "double fertilization" will be discussed in connection with the endosperm. But I wish to recall at this point Lotsy's (19) statement that both male cells function in the production of embryos, or, in other words, that twice the number of embryos are formed as there are entering pollen tubes. While this may be the case occasionally in *G. gnemon*, my sections show that in the great majority of cases only one functions as is usual. In most cases one fertilized egg only is found in a sac. Lotsy was probably led into error by the number of suspensors which are produced by a fertilized egg (page 44). The only evidence I have found in favor of Lotsy's statement is one case in which two fertilized eggs were present, although only one tube had apparently entered the sac. Whatever be the conditions in *G. gnemon*, certainly only one male cell usually functions in other species. I have often seen both male nuclei in a compartment with an egg and obviously the one which would not fertilize this egg could not fertilize any other.

10. Endosperm

As soon as the contents of a pollen tube enter the embryo-sac all the nuclei within the sac except the egg nuclei begin to divide. The divisions take place simultaneously throughout the sac. The similarity of the stages in all the dividing nuclei is remarkable. Figure 43 represents such a sac with all its nuclei in the same stage of division. The male nuclei can be seen entering at the left beneath the small second embryo-sac. Further down against the wall are two eggs. The divisions are repeated once or twice and with great rapidity. The nuclei which had all been large and loose in structure become greatly reduced in size and very dense as well as very numerous. In *G. gnemon* the subsequent events differ from those in other species and will be described separately.

(*a*) *G. gnemon*.—In this species although the parietal layer of protoplasm becomes thicker and encroaches on the vacuole, nevertheless the latter remains for a long time. In the thick band of protoplasm one finds a large number of small, deeply staining nuclei. The number is particularly large in the mass of protoplasm at the bottom of the sac and in that surrounding the egg. Then at the base of the sac walls appear in such a way as to form compartments. Each of the latter contains a mass of protoplasm and several (up to 10) nuclei. The walls are formed first at the extreme base of the sac and then gradually higher and higher up in the parietal layer of protoplasm. The result is a mass of cellular endosperm in the form of a shallow cup at the bottom and the parietal band of free-nucleated protoplasm above. In each cell are several nuclei. Figure 55 represents a sac in which walls are just beginning to form at the bottom, and figure 56, a later stage in which walls are forming higher up in the parietal protoplasm. It will be observed that each compartment is multinucleate.

The next step is the fusion of all the nuclei in each compartment into a single mass. The fusion takes place first in the lowermost compartments and then progressively in the higher ones. Consequently in certain sacs various stages in the process can be observed. Figure 57 represents a section of the base of a sac in which fusion has taken place in the lowermost compartments but not in the highest. As this section is tangential the vacuole in the center of the sac does not appear. Figure 58 shows a later stage in which fusion has



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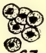

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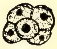

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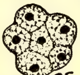

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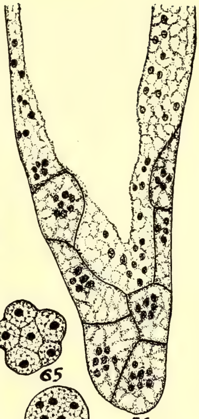

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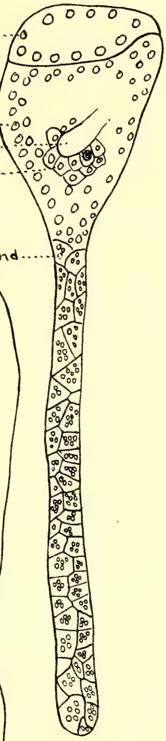
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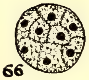

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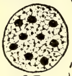

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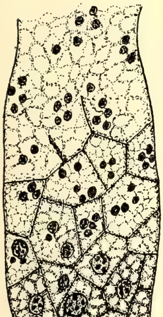

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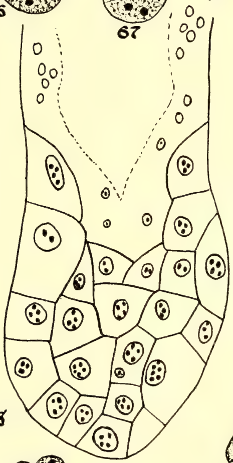

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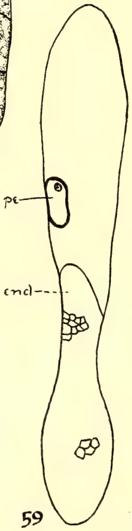

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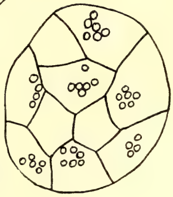

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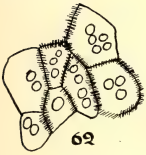

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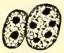

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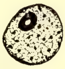

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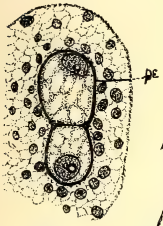
occurred in all the compartments. There results a mass of uninucleate cells with a more or less deep depression in the center (in the position of the vacuole). No more compartments are formed in this way. Indeed, even while fusion is occurring in the uppermost compartments division is taking place in the lowermost. Figure 53 shows a whole sac at this stage. At the bottom is the mass of uninucleate cells; above is the parietal layer of protoplasm with its free nuclei; and in the upper expanded part of the sac is the fertilized egg surrounded by a dense mass of protoplasm and nuclei.

Some stages in the fusion of the nuclei are represented in figures 63 to 69. Figure 63 shows the small nuclei grouped together. In figure 64 the membrane of the central nucleus is intact while those separating the other nuclei are disappearing and are represented by dotted lines. In figure 65 the outlines of the individual nuclei are still plain though the membranes between them are breaking down. In figure 66 the whole mass has lost its scalloped edge and has assumed more of the appearance of a single nucleus, although the boundary of each constituent nucleus is still visible. In figure 67 the individual nuclei are no longer distinguishable, although the nucleoli derived from them remain distinct. The presence of a large number of nucleoli in these fusion nuclei is characteristic for a considerable time. Sometimes all the nuclei in a compartment do not fuse into a single mass at once, but first form two or more masses which later fuse to one (fig. 68). Sometimes one small nucleus may remain distinct until all the others have fused and then be absorbed into the general mass. After a number of divisions of the fusion nucleus the numerous nucleoli (each derived from a constituent nucleus) disappear and a single larger one with the characteristic cavity in the center is found (see fig. 69). The number of nuclei which fuse appears to vary widely. I have never observed fewer than three nor more than ten.

The further growth of the endosperm takes place entirely in the cellular mass at the bottom. All the cells divide but the growth is most rapid at the extreme lower edge of the sac. Both cytoplasm and nuclei are much denser here than in the cells at the top of the endosperm (fig. 57). Owing to its more rapid growth the lower part of the endosperm at first becomes wider than the upper part. Gradually the tissue grows up the narrow part of the flask but always leaves the expanded part empty. The cavity can be seen with the naked



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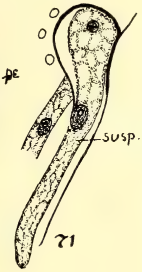

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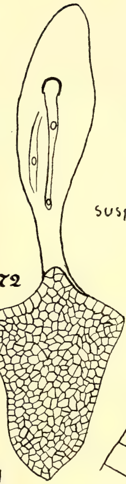

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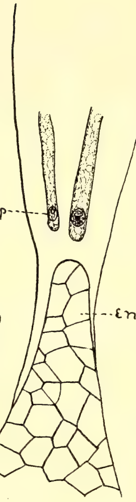

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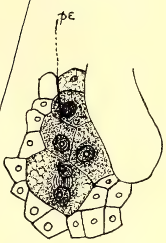

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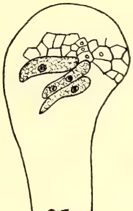

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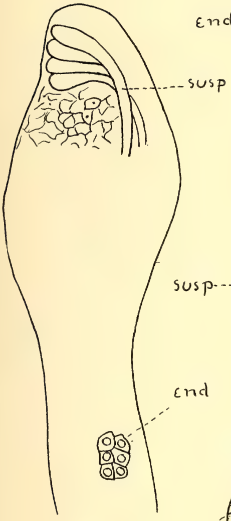

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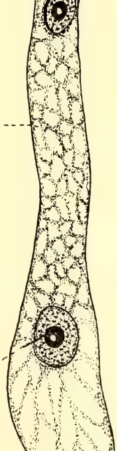

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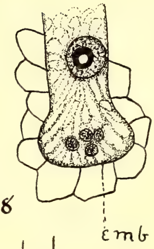

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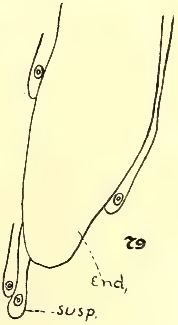

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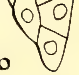

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eye in young fruits. Most of the growth takes place downwardly aned laterally. Figure 59 shows a whole sac with young endosperm at the bottom and the fertilized egg at the side. The endosperm never grows much higher than this. Figure 72 shows a later stage and one which is often found.

It is plain from this description that Lotsy's statement is correct that at a certain time there is a cellular mass at the bottom of the sac while there are only free nuclei above. Coulter (7) thought that Lotsy had mistaken the peculiar pavement tissue in the nucellus (page 21) for tissue within the sac. Lotsy was in error, however, in stating that this cellular tissue is present before the pollen tube enters. As we have seen it does not develop until the male cells are within the sac, though I am not prepared to say that compartments are not present before the actual fusion of egg and sperm has taken place. Not having seen the actual fertilization I cannot say with certainty at what stage of endosperm formation it takes place in this species. But I have frequently observed young fertilized eggs in sacs in which compartments had already formed. It seems that the formation of compartments and fusion of endosperm nuclei go on concurrently with fertilization. Lotsy thought that the female gametophyte of *G. gnemon* represented an intermediate condition between that of Gymnosperms and Angiosperms in that at fertilization time the endosperm was partly cellular and partly free-nucleated. Although this gametophyte does represent an intermediate condition, it is much more like that of Angiosperms than Lotsy believed, because when the pollen tube enters it contains only free nuclei and eggs.

The free nuclei in the upper part of the sac above the region of cell formation take no part in the production of endosperm. Except in the immediate vicinity of the fertilized egg they all disintegrate more or less rapidly. Those which surround the fertilized egg increase in number for a time and form a dense, deeply staining mass, which is well defined (fig. 53). I have occasionally observed the formation of compartments here similar to those at the bottom. The function of these nuclei and of the protoplasm in which they lie, appears to be the nourishment of the fertilized egg. But sooner or later these also degenerate leaving the fertilized egg alone in the cavity of the embryo-sac some distance above the endosperm.

(*b*) *G. sp. 33*.—In this species and similar ones endosperm formation takes place in a somewhat different manner. As soon as the pollen tube enters, the same rapid divisions occur throughout the sac and result in the production of a large number of small nuclei. But in these forms wall formation also takes place in the expanded part of the sac in the vicinity of the egg. It is particularly easy to observe it here. The spindle fibers of the last few divisions remain and consequently each nucleus is connected by fibers with several others. The walls are formed across these groups of fibers in the usual way. Not all the groups, however, are involved and consequently compartments are formed containing several nuclei (see fig. 62). The fibers not involved in wall formation disappear. The result is a group of multinucleate compartments in which fusion of the nuclei occurs as described for *G. gnemon*. The compartments are formed first in the vicinity of the egg and around the end of the pollen tube. Consequently at fertilization time one finds this group of cells surrounding the egg and male nuclei and in the remainder of the expanded part of the sac only free nuclei. The latter region becomes filled with cells partly by the division of those already present and partly by the formation of new compartments among the free nuclei.

Meanwhile endosperm has been forming down in the neck of the flask. Here too the process is not the same as in *G. gnemon*. The original divisions accompanied by increase in the cytoplasm, continue until the whole of this part of the sac becomes filled with cytoplasm and nuclei. In other words the vacuole becomes filled. It will be recalled that this part of the sac is much longer and narrower than in *G. gnemon*. The whole neck of the flask then becomes divided into compartments. Figure 60 shows this condition in longitudinal section and figure 61 in transverse section. I could not see any relation between division spindles and walls in this region; in fact the latter appeared to be more in the nature of cleavage walls. Each compartment contains more nuclei than in *G. gnemon*, but fusion takes place in the same way, resulting in the production of uninucleate cells.

At a certain stage in endosperm formation, therefore, one finds cellular tissue throughout the narrow part of the sac and around the fertilized egg in the expanded part. Elsewhere in latter region are free nuclei or vacuoles (see figs. 49 and 52). The whole sac later becomes filled with cells by the division of those already present and by the formation of new ones. Further growth takes place chiefly in the lower end of the sac. Consequently the form of the endosperm becomes reversed, the large end being below. The nucellus is gradually replaced. The originally expanded part of the sac enlarges very little, if any, and the nuclei and protoplasm within it disappear. The cells in this region become largely disorganized by the growth of the suspensors.

It should be pointed out that considerable development has taken place in this endosperm before fertilization occurs. While there is no cellular tissue in the sac at the entrance of the pollen tube, a considerable amount of it develops before the actual fusion of the sexual nuclei. In fact fertilization does not take place until the narrow part of the sac is filled with compartments and the group of cells have been formed in the expanded part of the sac.

It is evident that the process of endosperm formation in *G. sp. 33* is more primitive than that of *G. gnemon*. In the first place the whole sac of the former species becomes divided into cells as in typical Gymnosperms and all the nuclei contribute to endosperm formation, while in *G. gnemon* only a few of the nuclei contribute to endosperm formation. In the second place the development of endosperm in *G. sp. 33* has gone much farther before fertilization than in *G. gnemon*.

The important departures from the typical Gymnospermic method of endosperm formation are three in number: (1) The delay in cell formation until after the pollen tube has entered, (2) the fusion of nuclei in each cell, (3) the participation of only a few of the nuclei in endosperm formation (*G. gnemon*). Now all these departures from the Gymnospermic method are approaches to the Angiospermic method. They will be discussed separately.

1. In all other Gymnosperms a cellular endosperm is formed before the pollen tube enters the archegonium and the egg is fertilized. In Angiosperms the endosperm never forms until after the pollen tube enters the embryo-sac though it may begin to develop before actual fertilization takes place. In Gnetum one never finds endosperm before the entrance of the pollen tube. It is plain therefore that in this very important respect, Gnetum follows the Angiospermic method. But in *G. sp. 33* there is a reminiscence of the Gymnospermic method in that a considerable mass of endosperm is formed before the actual fusion of sexual nuclei occurs. In *G. gnemon* fertilization takes place sooner, though I have never seen a fertilized egg before a few cells were formed. In this connection it should be pointed out that in Casuarina, one of the lowest of the Angiosperms, Treub (31)

observed a considerable mass of endosperm before fertilization occurred.

2. In typical Gymnosperms there is nothing which resembles the fusion of nuclei which in Angiosperms precedes endosperm development. In typical Angiosperms two nuclei only of the female gametophyte, a micropylar and an antipodal, unite. In Gnetum several nuclei in each compartment unite. The chief differences from Angiosperms are (1) that many fusion nuclei result and (2) that several nuclei unite to form a single fusion nucleus. Nevertheless the essential fact remains that a fusion of nuclei occurs before the endosperm develops. And it should be remembered that in several primitive Angiosperms more than two nuclei unite. Thus in *Peperomia hispidula* Johnston (11) reports that 14 nuclei fuse to form the endosperm nucleus. Therefore one of the two differences between Gnetum and Angiosperms in this connection disappears (and it is significant that of all the Angiosperms, forms like Peperomia should most resemble Gnetum). The other difference, the number of fusion nuclei, loses its importance when we remember that in *G. gnemon*, there is a tendency to reduce the number [see (3) below]. Accordingly it seems a reasonable conclusion that the fusion of nuclei in Gnetum is really a forerunner of that in Angiosperms.

3. In Gymnosperms the whole female gametophyte is endosperm tissue. In Angiosperms endosperm formation involves only two nuclei of the female gametophyte. *G. sp. 33* resembles the Gymnosperms in that all the gametophytic nuclei contribute to endosperm formation. There are many fusion nuclei throughout the sac. But in *G. gnemon* only the nuclei at the bottom of the sac are concerned in endosperm formation. At most ten compartments with their fusion nuclei participate while all the rest of the sac (by far the larger part) has nothing to do with it. Within the genus itself, therefore, there is a marked tendency away from the Gymnospermic condition in *G. sp. 33* toward the more Angiosperm-like condition of *G. gnemon.*

The only other important difference between the endosperm of Angiosperms and that of Gymnosperms is one of which we find no trace in Gnetum, namely, the fertilization of a fusion nucleus by a male nucleus. No male nucleus is concerned with endosperm formation in any species of Gnetum. At this important point, therefore, the resemblance between Gnetum and the Angiosperms breaks down. In other words Gnetum does not throw much light on the origin of "double fertilization" or "triple fusion" and yet from the conditions in Gnetum one may well imagine how the phenomenon arose. It should be remembered that in the embryo-sac before fertilization there are fusion nuclei and a male nucleus in addition to the egg and male nucleus which will fuse to form the embryo. While I cannot confirm Lotsy's statement that the second male nucleus always functions, I have seen one case in which it did function. If the second male nucleus fertilized a fusion nucleus in place of an egg the typical Angiosperm condition would be reached. Furthermore it has been shown that in certain of the lower Angiosperms double fertilization does not occur.

We have just concluded that in respect to three of the four important differences between the endosperm of Gymnosperms and that of Angiosperms, Gnetum resembles the latter. And yet in regard to each of them we find evident reminiscences of the Gymnosperm condition. It seems, therefore, a reasonable conclusion that the endosperm of *Gnetum* is really the type from which that of Angiosperms has been derived, and that it in turn has been derived from the Gymnosperm type.

Moreover the conditions in Gnetum have a distinct bearing on the morphology of the endosperm in Angiosperms. As is well known there are two views on this subject: (1) that it is belated gametophytic tissue and (2) that it is really an embryo rendered monstrous by the introduction of the second female nucleus. In Gnetum there can be no doubt that it is belated female gametophyte, although the fusion of nuclei preceding its initiation is difficult to understand. Indeed, although the conditions are much more primitive in Gnetum we appear to be no nearer an understanding of the meaning of the fusion than we are in the case of Angiosperms. We can only fall back on the old idea that it is in the nature of a vegetative stimulus to growth. Now in spite of the fertilization of the fusion nucleus by the second male nucleus in a great many Angiosperms, the endosperm of the latter group is of the same nature as that of Gnetum. This is true not only because of the transitions seen in Gnetum but also because the triple fusion is not always a prerequisite to endosperm formation in Angiosperms. It follows therefore that the endosperm of Angiosperms is just as much female gametophyte as is that of Gnetum and this would be true whether or not the endosperm of Angiosperms had been derived from that of Gnetum.

11. EMBRYO

The nucleus of the fertilized egg is large and dense. Its nucleolus is extremely large and has a distinct cavity in the center. In *G. gnemon* the fertilized egg is surrounded by a mass of protoplasm and free nuclei (see figure 54), while in *G. sp. 33* it is surrounded by cells of the endosperm (figure 51). The development of the embryo in *G. sp. 33* will be described first.

The fertilized egg divides two or three times and produces a small group of cells. These divisions are accompanied by wall formation. There appears to be no definite order in the divisions and no definite arrangement of the cells. This pro-embryo can usually be readily distinguished from the surrounding endosperm cells by the density of the protoplasm and size of the nuclei and nucleoli. Figure 74 represents such a pro-embryo against the end of the pollen tube. Each cell then elongates to form a suspensor (figures 75 and 76) which penetrates far towards the bottom of the endosperm. (It will be recalled that most of the growth of the endosperm occurs in the lower part of the sac.) These suspensors are very difficult to follow and consequently it is hard to determine their nuclear condition with certainty. In almost all cases one can find only a single enormously large nucleus in each. I have, however, seen newly forming suspensors with two nuclei and in *G. moluccense* I have seen two large nuclei near the end of an elongated suspensor. On account of the difficulty of tracing the tortuous suspensors through their whole length, however, it may be that some nuclei were overlooked. But in any case very few nuclei are present, probably only one normally. If more are formed they must degenerate very quickly.[1] In the much elongated suspensors the common appearance is a great length of empty tube with a mass of deeply staining protoplasm and a huge nucleus only at the end. So far as I have observed walls are never formed in this suspensor, and there is very little branching.

The later stages of embryogeny I have seen only in *G. moluccense* and in *G. funiculare* but as in most other respects these species resemble *G. sp. 33* it is altogether likely that in the latter species the conditions are similar. In *G. moluccense* the suspensors are very often found outside the endosperm. They grow either through the latter or just between it and the nucellus. Sometimes they grow even beyond the endosperm into the tissue at the base of the nucellus. Figure 97 represents such a condition. I have never seen an embyro developing in this position, they were always within the endosperm. Before the embryo proper is formed the end of the suspensor enlarges and in the next stage that I have observed this swollen end contained four small nuclei (see figure 78). There was no wall separating the swollen end from the rest of the tube. From this structure the embryo is organized in a manner not determined. A young embryo consisting of a small group of cells irregularly arranged is shown in figure 80.

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[1] I regret that in my preliminary note (28) I gave the impression that many nuclei were present.

In *G. gnemon* the pro-embryo differs from that of the other species in that only two cells are formed before the development of suspensors. The two-celled stage is shown in figure 70. I cannot state whether or not the dividing wall develops in connection with the division of the nucleus or is in the nature of a cleavage wall. Both cells develop into suspensors (figure 71), which grow down through the empty upper part of the sac to the developing endosperm which they penetrate. Figure 72 represents the suspensors growing towards the endosperm and figure 73 the same thing under greater magnification. I have not observed the later stages in this species.

This account of the embryogeny of *G. gnemon* differs from that of Lotsy in regard to the division of the fertilized egg. Lotsy states that the fertilized egg itself elongates without division to form the suspensor. The explanation of the discrepancy in the two accounts is likely to be found in Lotsy's statement that both male cells always function in fertilization. Apparently what Lotsy considered to be two fertilized eggs, I have called a two-celled proembryo. In support of my view I would call attention to three points: (1) I have more than once observed one fertilized egg alone in a sac (this may, however, be interpreted as the failure of one of the male cells to function; but in that case, the statement that both male cells always function is incorrect); (2) the two cells are always in close contact and have every appearance of having resulted from the division of a single cell; (3) in *G. sp. 33* several cells are undoubtedly formed before the suspensors elongate.

Coulter (7) states that the suspensor within the endosperm is divided by transverse cleavage walls. While this may be true within the endosperm I have observed no such walls before the suspensor reaches that tissue. Furthermore in the other species no such walls were observed even in the endosperm. Coulter's account follows the embryo-formation farther than I have done, showing that the multinucleate terminal cell becomes divided up by cleavage walls into uninucleate cells.

The most important point about this process of embryo formation is that the free nuclear divisions characteristic of Gymnosperms are eliminated. Division of the fertilized egg in *G. sp. 33* at least, is accompanied by wall formation as in Angiosperms. In another important respect therefore Gnetum takes its place with the Angiosperms. This elimination of the free nuclear stage was foreshadowed in Welwitschia (Pearson, 21) in which only two free nuclei are formed and are then separated by a cleavage wall.

12. General Conclusions

The significance of the conditions found in Gnetum has been discussed in connection with the descriptions of the various structures examined. It remains to point out certain general conclusions which may be deduced from a consideration of the evidence as a whole. The conclusions are in respect to the relationships of the Gnetales (*a*) to each other, (*b*) to the Gymnosperms, (*c*) to the Angiosperms and also in respect to the origin of Angiosperms.

(*a*) The evidence shows that the three genera of the Gnetales are widely different in many morphological points and yet are really phylogenetically related. In habit, anatomy, gametophytic structure, endosperm and embryo formation the genera are all very different. Nevertheless in regard to each of these subjects there are essential points which indicate a common phylogeny. For example, the occurrence in the flowering axis of Gnetum of a type of vessel found elsewhere only in Ephedra can be explained only on the basis of a phylogenetic connection. Other examples are the similarity in flower structure and in the early stages of embryo formation, although the details vary greatly in the three genera. If all three are really related it follows from their present great differences that the Gnetales must formerly have been a very large and diversified group. While the three genera can by no means be arranged in a series according to the degree of specialization it is clear that Gnetum is in many respects the most advanced, that in a few respects Welwitschia has progressed furthest, and that Ephedra is nearest to the ancestral Gymnosperm stock.

(*b*) The conditions in Gnetum throw little direct light on the problem of the Gymnospermic relationship of the Gnetales. Being in many respects the most specialized member of the group, it presents little evidence not already revealed in the case of Ephedra and Welwitschia. The evidence concerning the Gymnospermic relationship must naturally be sought in the most primitive member of the group, the genus Ephedra. That evidence has been presented from the gametophytic standpoint by Land (15, 16) and from the anatomical standpoint by the writer (27). From both fields it indicates definitely that the relationship is with the Coniferous Gymnosperms. It is, however, difficult to understand how the primitive bisporangiate Gnetalean flower can have been evolved from anything found in the Coniferales. But there is nothing found elsewhere in the Gymnosperms which offers an easier solution of the origin of this flower.

(*c*) In regard to the Angiospermic relationship it will be recalled that almost every structure described in the preceding pages shows some approach to the Angiospermic condition and that some structures show conditions almost completely Angiospermic. The more important points are: the form of the inflorescence, particularly of the abnormal ones, the arrangement of the parts of the flower, the presence of an ovary with a style, the form of the stamen, the germination of the microspores at some distance from the nucellus, the behavior of the stalk cell, the free-nucleated embryo-sac and absence of archegonia, the organization of eggs, the fusion of nuclei preceding endosperm formation, the reduction in the number of endosperm producing cells, and the absence of free nuclear divisions in the proembryo. That the anatomy is just as clearly Angiospermic is evident from the possession of vessels, broad rays, and companion-cells in the bast. The habit of course is completely Angiospermic. Such a body of evidence can scarcely be ignored or put aside as the result of parallel development. Indeed in applying most of the contrasts ordinarily employed to distinguish Angiosperms from Gymnosperms, it is found that Gnetum would be classified with the higher group. Accordingly the sum of the evidence from all sides seems to lead to the conclusion that Angiosperms are phylogenetically related to Gnetales. This does not mean that any modern member of the Gnetales represents the type from which Angiosperms were derived but that the ancestors of Angiosperms were not far removed from the genus Gnetum.

The only group of plants which rivals the Gnetales in the claim on the ancestry of the Angiosperms is the Mesozoic Bennetitales. Their claims have been advocated by Arber and Parkin (1) and by Scott (24). According to the latter author they are three in number: (1) the organization of strobili on the same plan as in typical Angiosperm flowers (2) the formation of a fruit enclosing the seeds (3) the exalbuminous nature of the seeds. It is admitted that these are the only points whether of phylogenetic significance or not in which the Bennetitales resemble Angiosperms. In regard to the exalbuminous nature of the seeds it need only be remarked that by no means all Angiosperms have such seeds and certainly this is not the case in many primitive forms. In regard to the formation of fruit enclosing the seeds the claims of Gnetum are even stronger for the fruit of the latter is formed in a much more typically Angiospermic fashion. The organization of the strobili while resembling that of an Angiosperm flower in regard to the arrangement of the mega- and microsporangia is widely different in many important respects: The ovules are stalked; there is nothing resembling a carpel (a most important point); there are sterile scales between the ovules; the microsporophylls with their fern-like branching and numerous sporangia are as different as possible from the Angiosperm stamen. Moreover if we conclude that the Angiosperm flower of the Magnolia type has been derived from the Bennetitales strobilus we encounter the insurmountable difficulty of explaining the occurrence of the simple catkin-like inflorescence on forms admittedly primitive. On the other hand these catkin-like inflorescences relate themselves strikingly to the strobili of Gnetum.

Apart from the organization of the strobili there are many points which seem to preclude the possibility of the Bennetitalean ancestry of the Angiosperms. Some of them are: the Cycadean habit and leaves, all the essential anatomical points (see Thompson, 27), undoubted possession of motile spermatozoa, primitive Gymnosperm condition and absence of anything foreshadowing the Angiosperm condition in gametophytes, endosperm or embryo. Now in all these respects, as has been shown, Gnetum approaches the Angiosperm conditions and in many of them has actually reached those conditions. As between the Bennetitales and Gnetales, therefore, the decision must surely be made in favor of the latter group. Nor is it sufficient to assume with Arber and Parkin (2) that the Gnetales and Angiosperms developed in parallel lines from the Bennetitales. Aside from the argument that the points just mentioned preclude any possibility of the Bennetitalean ancestry of Angiosperms, the great resemblance between the latter group and the Gnetales are not satisfied by any such theory. Moreover the evidence relates the Gnetales, not to the Bennetitales but to the Conifers.

It remains to remark that the Amentales are the Angiosperms which most closely resemble the Gnetales and appear therefore to be the most primitive order of the phylum. There is the possibility that both Gnetales and Amentales are somewhat reduced as is indicated by the flower structure of the former.

13. SUMMARY

1. *Actual Conditions.*—Abnormal strobili are found in which the flowers are arranged in a spiral, the whole resembling very much a catkin of the Amentales. Vessels of the Ephedra type are present in the axis of the strobilus.

The development of the microsporangium takes place in the usual way. Two layers of parietal cells are formed. There is no endothecium. The tapetum is developed from sporogenous cells. The period of development is very short.

In the development of the megasporangium the three envelopes arise in acropetal succession. The style develops conducting tissue. Two to four mother cells are formed, two or three produce megaspores, and two or three megaspores develop into embryo-sacs.

The usually abortive ovules of the staminate strobili frequently possess embryo-sacs and may produce seeds.

In the male gametophyte no prothallial cells are formed; the microspores frequently germinate in the style at a distance from the nucellus; the stalk cell never passes into the pollen tube; distinct male cells are formed while the tube is on the nucellus.

The female gametophyte develops in typical Gymnospermic fashion until approximately 256 free nuclei are present in *G. gnemon* and 512 in other species. No cell walls are formed before the entrance of the pollen tube. Definite eggs are organized. Two or three gametophytes usually develop in each ovule.

After the entrance of the pollen tube rapid divisions occur in the female gametophyte. Multinucleate compartments are then formed and all the nuclei in each compartment fuse. The further growth of the endosperm occurs by the division of these compartments. In *G. gnemon* only a few fusion nuclei participate in endosperm formation.

In *G. sp. 33* at least fertilization is delayed until after a considerable amount of endosperm is formed.

The fertilized egg divides to form two cells in *G. gnemon* and several cells in other species. Each of these elongates to form a suspensor from the end of which an embryo is formed.

2. *Inferences.*—The strobili of Gnetum are closely related to the catkins of Amentales.

The flowers of Gnetum are reduced from a bisporangiate condition with parts arranged as in typical Angiosperm flowers.

The inner envelope of the ovulate flower is an ovary homologous with that of Angiosperms and bearing a true style.

The outer envelopes are in the nature of a perianth.

The abortive ovulate flowers are homologous in every respect with the fertile flowers.

The male gametophyte is typically Angiospermic except that it has an evanescent stalk cell.

The female gametophyte is typically Gymnospermic in the early stages but distinctly Angiospermic in the later ones.

The fusion of nuclei preceding endosperm formation is a forerunner of that in Angiosperms.

It is not possible to relate the structures of an Angiosperm embyro-sac to archegonia.

The endosperm of Angiosperms is best interpreted as gametophytic tissue.

The proembryo is Angiospermic.

The different genera of the Gnetales are widely different in morphology and yet are phyogenetically connected.

The Angiosperms have been derived from ancestors very much like modern Gnetales. In fact the genus *Gnetum* should probably be classified with Angiosperms.

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16. EXPLANATION OF PLATES II—VII

PLATE II

FIG. 1. *Gnetum funiculare.* Abnormal spiral strobilus. *c.*, collar; *f.*, ovulate flower. × 2.

FIG. 2. *G. gnemon.* Abnormal staminate strobilus with many abortive ovulate flowers. *f.*, ovulate flower; *m.*, place of attachment of staminate flowers which have fallen. × 2.

FIG. 3. *G. gnemon.* Part of similar strobilus at a younger stage. *F.*, ovulate flowers; *m.*, staminate flowers. × 5.

FIG. 4. *G. gnemon.* Radial section of group of young flowers from staminate strobilus. *f.*, abortive ovulate flower; *pe.*, perianth; *r.*, central rudiment of staminate flower; *c.*, collar. × 200.

FIG. 5. *G. gnemon.* Similar section of older group. *m.*, microspore mother cells. × 200.

FIG. 6. *G. gnemon.* Young microsporangium. *e.*, epidermis; *a.*, archesporium. × 400.

FIG. 7. *G. gnemon.* Later stage. *e.*, epidermis; *p.*, primary parietal layer; *s.*, sporogenous cells. × 400.

FIG. 8. *G. gnemon.* Later stage. *p.o.*, outer layer of parietal cells; *p.i.*, inner layer. × 400.

FIG. 9. *G. gnemon.* Microsporangium. *ta.*, tapetum; *m.*, mother cells. × 400.

FIG. 10. *G. gnemon.* Microsporangium in stage of tetrad divisions. Letters as before. × 500.

FIG. 11. *G. sp. 33.* Young ovulate flower. *pe.*, perianth. × 100.

FIG. 12. *G. sp. 33.* Young ovulate flower. *o.i.*, outer integument; *i.i.*, inner integument. × 100.

FIG. 13. *G. sp. 33.* Later stage of ovulate flower. *h.*, hairs. × 50.

FIG. 14. *G. sp. 33.* Later stage. *i.i.*, inner integument elongating to form style; *s.*, embryo-sac. × 100.

FIG. 15. *G. sp. 33.* Mature ovulate flower. *pe.*, perianth; *st.*, style; *cav.*, cavity in style; *o.i.*, outer integument; *i.i.*, inner integument; *n.*, nucellus; *s.*, embryo-sac. × 50.

FIG. 16. *Peperomia sp.* Young ovulate flower. *ca.*, carpel. × 50.

PLATE III

FIG. 17. *G. sp. 33.* Longitudinal section of style. *n.*, nutritive layer; *p.*, pollen grains; *p.t.*, pollen tube. × 300.

FIG. 18. *G. sp. 33.* Style. Later stage. Lettering as before. Shows disintegration of nutritive layer. × 320.

FIG. 19. *G. sp. 33.* Young megasporangium. *e.*, epidermis; *a.*, archegonium. × 550.

FIG. 20. *G. sp. 33.* Young megasporangium. *p.*, parietal cells; *m.*, mother cells. × 550.

FIG. 21. *G. sp. 33.* Young megasporangium. Later stage showing one parietal cell divided. × 550.

FIG. 22. *G. sp. 33.* Young megasporangium. Transverse section. × 550.

FIG. 23. *G. sp. 33.* Young megasporangium, showing two mother cells, one of which is dividing. × 550.

FIG. 24. *G. sp. 33.* Young megasporangium, showing one mother cell undivided and two which have divided and produced daughter cells (*d*). × 550.

FIG. 25. *G. sp. 33.* Megaspores and four-nucleate sac. × 550.

FIG. 26. *G. gnemon.* Longitudinal section of young abortive ovulate flower from staminate strobilus. *pe.*, perianth; *n.*, nucellus; *i.i.*, inner integument; *o.i.*, rudimentary outer integument; *s.*, embryo-sac. × 200.

FIG. 27. *G. gnemon.* Transverse section of abortive ovulate flower. *v.b.*, vascular bundles. × 50.

FIG. 28. *G. gnemon.* Young microspore. × 1200.

FIG. 29. *G. gnemon.* Microspore. *t.*, tube nucleus; *g.*, generative cell. × 1200.

FIG. 30. *G. gnemon.* Microspore. Pollination stage. *t.*, tube nucleus; *b.*, body; *st.*, stalk cell. × 1200.

FIG. 31. *G. gnemon.* Microspore. Stalk and generative nuclei in a common cytoplasm. × 1200.

FIG. 32. *G. sp. 33.* Germinated microspore. × 550.

FIG. 33. *G. sp. 33.* Pollen tube entering nucellus. *b.*, body cell; *t.*, tube nucleus; *n.*, nucellus. × 550.

FIG. 34. *G. sp. 33.* End of pollen tube. *m.*, male nuclei; *t.*, tube nucleus. × 800.

FIG. 35. *G. sp. 33. G. sp. 33.* Tip of nucellus with pollen grains and tubes. *st.*, stalk nucleus; *p.t.*, pollen tube; *n.*, nucellus. × 300.

PLATE IV

FIG. 36. *G. gnemon.* Young embryo-sac. × 430.

FIG. 37. *G. gnemon.* Two young embryo-sacs from same nucellus. × 430.

FIG. 38. *G. gnemon.* Nearly mature embryo-sac showing mass of protoplasm and nuclei (*m.*) at bottom. × 200.

FIG. 39. *G. sp. 33.* Mature embryo-sac and small abortive one. × 75.

FIG. 40. *G. gnemon.* Top of embryo-sac and pollen tube. *e.*, egg; *t.*, tube nucleus; *m.*, male nuclei. × 400.

FIG. 41. *G. gnemon.* Whole embryo-sac with entering pollen tubes. Lettering as before. × 100.

FIG. 42. *G. gnemon.* Top of embryo-sac with entering pollen tube. × 400.

FIG. 43. *G. sp. 33.* Embryo-sac with entering pollen tube showing nuclei all dividing. *p.t.*, pollen tube; *s.a.*, abortive sac; *d.*, dividing nucleus; *e.*, egg. × 300.

FIG. 44. *G. gnemon.* Embryo-sac into which pollen tube has penetrated some distance. × 300.

FIG. 45. *G. sp. 33.* Embryo-sac just prior to fertilization. *e.*, egg; *m.*, male nuclei; *end.*, cells of endosperm. × 400.

FIG. 46. *G. gnemon.* Nucleus of female gametophyte. × 1200.

FIG. 47. *G. gnemon.* Egg nucleus. × 1200.

FIG. 48. *G. sp. 33.* Male nucleus. × 1200.

PLATE V

FIG. 49. *G. sp. 33.* Embryo-sac at fertilization time. *t.*, tube nucleus; *end.*, endosperm; *s.a.*, abortive sac. × 215.

FIG. 50. *G. sp. 33.* Sexual nuclei in contact. *e.*, egg; *m.*, male cells. × 400.

FIG. 51. *G. sp. 33.* Sac with fertilized egg. *f.e.*, fertilized eggs; *end.*, endosperm. × 400.

FIG. 52. *G. sp. 33.* Sac with fertilized egg. Earlier stage, showing many free nuclei. × 300.

FIG. 53. *G. gnemon.* Whole embryo-sac with fertilized egg. *f. e.*, fert. egg; *p.*, mass of protoplasm and nuclei surrounding egg; *end.*, endosperm; *n.*, free nuclei. × 100.

FIG. 54. *G. gnemon.* Fertilized egg surrounded by mass of protoplasm and nuclei. × 400.

PLATE VI

FIG. 55. *G. gnemon.* Base of embryo-sac showing beginning of endosperm formation. *w.*, newly formed wall. × 300.

FIG. 56. *G. gnemon.* Multi-nucleate compartments in endosperm formation. × 300.

FIG. 57. *G. gnemon.* Endosperm with partly uninucleate and partly multinucleate compartments. × 300.

FIG. 58. *G. gnemon.* Endosperm with uninucleate cells only. A slight amount of growth has taken place by division. × 300.

FIG. 59. *G. gnemon.* Whole embryo-sac with young endosperm. *p.e.*, proembryo. × 100.

FIG. 60. *G. sp. 33.* Whole embryo-sac with young endosperm. *s.a.*, abortive embryo-sac; *n.*, free nuclei; *p.t.*, pollen tube; *end.*, endosperm. × 100.

FIG. 61. *G. sp. 33.* Transverse section of embryo-sac with multinucleate endosperm. × 300.

FIG. 62. *G. sp. 33.* Wall formation in endosperm. × 550.

FIG. 63. *G. gnemon.* Endosperm nuclei massed before fusion. × 800.

FIG. 64. *G. gnemon.* Fusion of endosperm nuclei. Disappearing walls represented by dotted lines. × 800.

FIG. 65. *G. gnemon.* The same; later stage. × 800.

FIG. 66. *G. gnemon.* The same. Later stage. × 800.

FIG. 67. *G. gnemon.* The same. Later stage. Shows the numerous nucleoli. × 800.

FIG. 68. *G. gnemon.* The same. Formation of two masses which fuse later. × 800.

FIG. 69. *G. gnemon.* Endosperm nucleus after several divisions. × 800.

PLATE VII

FIG. 70. *G. gnemon.* Pro-embryo of two cells surrounded by protoplasm and nuclei. × 300.

FIG. 71. *G. gnemon.* Suspensors developing from pro-embryonal cells. × 300.

FIG. 72. *G. gnemon.* Suspensors growing towards endosperm. × 50.

FIG. 73. *G. gnemon.* The same; greater magnification. × 300.

FIG. 74. *G. sp. 33.* Pro-embryo of several cells. × 400.

FIG. 75. *G. sp. 33.* Suspensors developing from pro-embryonal cells. × 200.

FIG. 76. *G. sp. 33.* Old suspensors in embryo-sac. × 100.

FIG. 77. *G. moluccense.* End of suspensor with two large nuclei. × 200.

FIG. 78. *G. moluccense.* End of suspensor showing four nuclei which will give rise to embryo. × 200.

FIG. 79. *G. moluccense.* Suspensors outside of and below endosperm. × 100.

FIG. 80. *G. moluccense.* Young embryo. × 50.

APPENDIX

The following list includes the names of the species used together with the name authorities:

Gnetum Gnemon L.
Gnetum latifolium Blume
Gnetum moluccense Miquel
Gnetum neglectum Blue
Gnetum ula Brongniart
Gnetum sp. Borneo—Buitenzorg Garden Records
Gnetum sp. 33—Buitenzorg Garden Records
Gnetum sp. 59—Buitenzorg Garden Records

NOTES ON THE DISTRIBUTION AND GROWTH OF NORTH DAKOTA CUSCUTAE

O. A. Stevens

Plants of the genus Cuscuta, collected during the past year, led to a revision of the specimens in the North Dakota Agricultural College herbarium, and the list is here given. Dr. J. Lunell of Leeds, N. D., who has the only other extensive herbarium within the state, has kindly loaned me his material of the group.

Cuscuta epilinum Weihe. Commonly attributed to the state, but we have no record of its occurrence; nor has the examination of several thousand samples of flax seed, a large part of them uncleaned, shown any trace of the seeds.

Cuscuta epithymum Murr. Not represented, but was found on white clover in a lawn at Fargo in 1912 or 1913. Seeds of this dodder have been found in several samples of white clover seed.

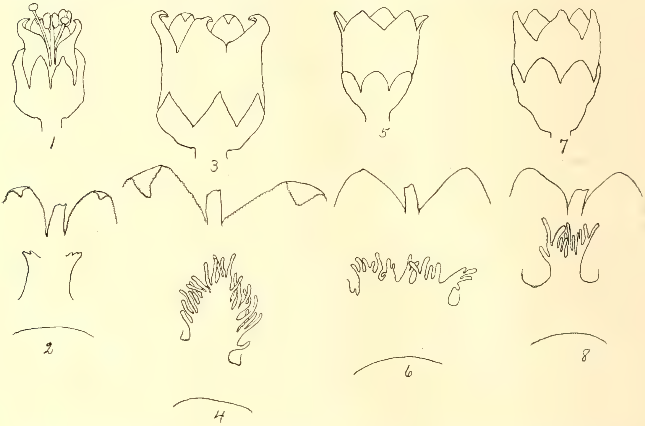
Cuscuta planiflora Tenore. Not definitely recorded, but seeds were found in samples of alfalfa seed from near the western line of the state.

Cuscuta arvensis Beyrich. Fargo, Valley City, Minot, and Logan Co. Two or three instances of its presence in alfalfa fields have come to notice. One collection by the writer, at Fargo, was made along the river bank where it grew upon everything within reach (25 spp.), including such riparian plants as *Xanthium commune*, *Polygonum lapathifolium*, *P. emersum*, *Mentha canadensis*, *Lycopus americanus*, *Stachys palustris*, *Bidens vulgata*, *Mimulus ringens*, and *Scutellaria lateriflora*. I found this dodder at Valley City, growing on *Kuhnistera oligophylla*, *Chamaerhodos erecta* and *Ambrosia psilostachya* in an old sand pit on the hillside.

Cuscuta indecora Choisy. Fort Totten, on *Lotus americanus*.

Cuscuta coryli Engelm. Numerous localities through the eastern and northern part of the state are represented, and the following hosts: *Solidago serotina*, *S. canadensis*, *Aster paniculatus*, *Helianthus tuberosus*, *Chenopodium album*, *Monarda fistulosa* and *Medicago sativa*. I have also observed it on shrubs, *Salix spp.*, *Rhus rydbergii*, *Rosa sp.*, and *Parthenocissus quinquefolia*.

Our specimens of *C. coryli* have been confused with other species, usually *C. gronovii* Willd. In most of the manuals the capsule is described as "pointed." Small,[1] however, gives "much depressed," which agrees with our plants. The size of the capsules and shape of the calyx lobes are quite variable. The fruiting clusters vary from a few capsules to loose, long-stalked cymes or dense masses 1 to 3 inches thick. Specimens collected at Fargo and also some grown in the garden were examined by Mr. F. H. Hillman, of the U. S. Department of Agriculture.



Text-figures 1, 3, 5, 7, of flowers × about 5, and of portion of corolla (figs. 2, 4, 6, 8) showing one scale × about 10; the latter drawn from camera lucida sketch. Flowers from preserved material except 1 and 2.

1–2. *Cuscuta coryli.* 3–4. *Cuscuta indecora.* 5–6. *Cuscuta plattensis.* 7–8. *Cuscuta gronovii.*

CUSCUTA CEPHALANTHI Engelm. Walhalla; also from Towner (Lunell) on *Artemisia frigida.*

CUSCUTA PLATTENSIS A. Nels. Lisbon, Jamestown, Leeds, Pleasant Lake, Turtle Mts., and Minot. On *Solidago canadensis, S. serotina, Aster paniculatus, Artemisia gnaphalodes, Steironema ciliatum, Convolvulus sepium, Lathyrus palustris, Clematis virginiana, Humulus lupulus, Symphoricarpos occidentalis* and *Salix cordata.*

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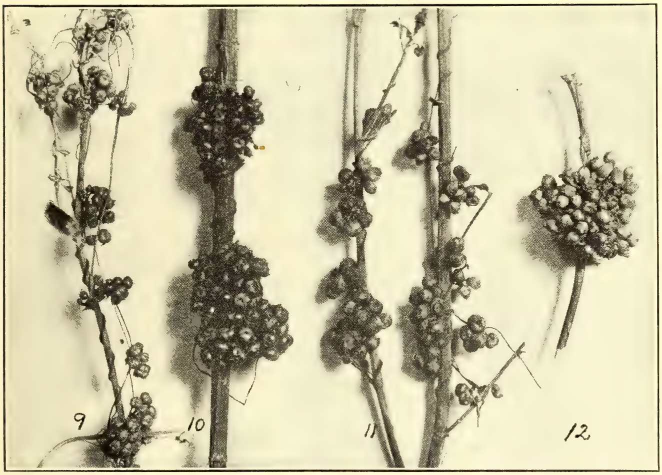
[1] Small, J. K., Flora of the Southeastern United States, p. 968, 1903.

CUSCUTA GRONOVII Willd. Fargo, on *Solidago canadensis, Sanicula marylandica, Deringia canadensis, Urtica gracilis* and *Urticastrum divaricatum.*

C. plattensis seems closely related to *C. gronovii* which it replaces west of the Red River valley, according to material at hand. Its habitat is the same as that of *C. coryli* (shrubs and coarse herbs along river banks, etc.), in fact, the two species were mixed in two sheets examined. The corolla lobes of *C. plattensis* and *C. gronovii* become more widely spreading or reflexed in older stages, so that a cluster of flowers is likely to give a somewhat different impression from the figures shown.



Text figures 9–12 of dry capsules about one-half natural size. (Photo by W. C. Palmer).

9. *Cuscuta coryli* on *Medicago sativa.* 10. Same on *Helianthus tuberosus.* 11. *Cuscuta plattensis* on *Aster paniculatus.* 12. *Cuscuta gronovii.*

The presence of *C. coryli* on alfalfa has not been reported previously and is of special interest. The first record for it was obtained in 1914 by planting some seeds taken from a sample of alfalfa said to have come from Pierre, S. D. In this sample there were about 300 Cuscuta seeds per ounce. A number of seed samples have indicated the presence of this species in alfalfa fields along the Missouri river in this state. Some search of such fields in 1914 and 1915 disclosed only an occasional plant. The economic status of *C. coryli* is therefore open to further investigation. One plant in the garden produced about 7,000 seeds, although the species does not seem to produce as much seed as *C. arvensis*. The seeds are *quite similar* to those of *C. indecora*, *plattensis*, and *gronovii*. In cleaning the seed grown from the South Dakota sample it was found that about half of the Cuscuta could be screened from the alfalfa seed on account of the larger size of the former. The following measurements were made with a binocular microscope at 50 diameters.

SIZE OF SEEDS OF CUSCUTA

Species	Minimum	Maximum	Av. of 50
C. planiflora	.9 × .6 mm.	1.2 × .8 mm.	1.00 × .73 mm.
C. arvensis	.9 × .9	1.6 × 1.3	1.33 × 1.15
C. coryli	1.4 × 1.3	2.6 × 2.1	1.80 × 1.50
C. gronovii	1.4 × 1.4	2.4 × 1.8	1.90 × 1.60
C. plattensis	1.6 × 1.3	2.5 × 2.1	2.10 × 1.70

From plots in the garden the germination of scattered Cuscuta seeds was observed as early as April 30, but little growth was made until the last of June and flowering began about the second week in August. *Cuscuta coryli* was killed by frost a little earlier than the *C. arvensis* in the same plot.

SEED LABORATORY,
NORTH DAKOTA AGRICULTURAL EXPERIMENT STATION

LYSICHITON CAMTSCHATCENSE (L) SCHOTT, AND ITS BEHAVIOR IN SPHAGNUM BOGS

Göte Turesson

Lysichiton camtschatcense or the western skunk cabbage can undoubtedly lay claim to greater notoriety than any other herb of the Puget Sound region and the Vancouver strip. Being the only local representative of the aroids and endowed with characteristics strikingly aroidal in nature, it naturally arouses the curiosity of the botanist as well as of the layman. In early spring before other plants show sign of life the pale yellowish color of its peculiar spadix and spathe give life to the dull color of the dormant vegetation. The pleasure, however, is universely proportional to the nearness of the plant. Similar to its eastern congener, the western skunk cabbage enjoys by right the reputation of emitting a stench, comparable in nauseousness to that of a skunk's secretion. Although it is difficult to designate the exact "shading" of the smell, it is an illustrative example of what Kerner (14) in his odor scheme called indoloid. The intensity of the smell is greatest during the period of flowering, and thousands of Staphylinids and carrion flies swarm at this time around the swamps and marshes, attracted by the pungent odor of the skunk cabbage. Later in the season it again attracts the attention by the gigantic size of its leaves, in this region surpassing one meter in length and half a meter in width.

Being typically a plant of swampy places, stream margins and ditches, *Lysichiton camtschatcense* is sometimes found growing in sphagnum bogs under very peculiar circumstances. The clue which *Lysichiton camtschatcense* gives to the interpretation of the physiographic history of one of these bogs will be dealt with below. However, before taking up for discussion the rôle it plays in the past and present history of the bog, it becomes necessary to consider shortly some of its more typical habitats.

The plant societies in which Lysichiton is more or less conspicuous can be grouped in two series: the river series and the pond-swamp series (Cowles, 6). We find Lysichiton present already in the initial stages of the river, *e. g.*, in the ravine. Wherever the topography in the gully admits water to remain during the earlier part of the vegetative season the conditions are favorable for the establishing of Lysichiton. Water-pockets of about three meters' circumference and located in the bottom of a ravine at Maltby, north of Lake Washington, State of Washington, showed the following vegetation (April 1915): *Lysichiton camtschatcense*,[1] *Cardamine pennsylvanica*, *Ranunculus bongardi greenei*. The pools were partly filled with humous material slid down from the slopes together with leaves of the trees in the immediate vicinity, *Acer macrophyllum*, *Alnus oregona* and *Rhamnus purshiana*.

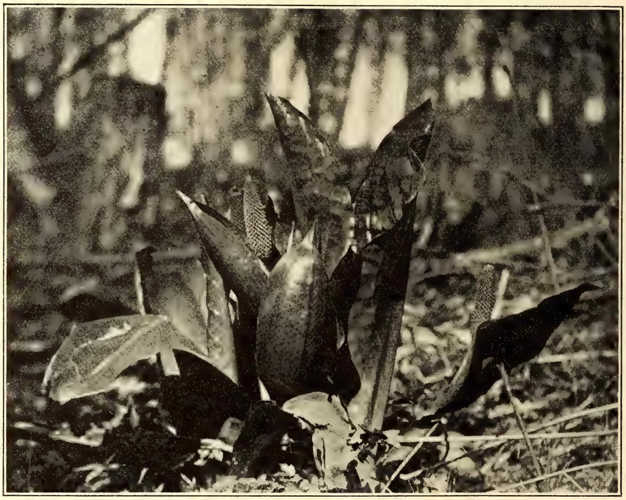


Fig. 1. *Lysichiton camtschatcense* at the time of flowering. Author's photograph.

With the appearance of a permanent stream in the ravine the conditions become still more favorable for Lysichiton. It is true, the erosive action of the stream at first prevents vegetation from gaining a foothold on the streambed, but as the energy of the current is slackened by the accumulation of debris and temporary floodings occur in the wet seasons, a characteristic brookside flora soon appears. *Lysichiton camtschatcense* plays a prominent part in such situations in this region. A representative area from a ravine of the above-mentioned nature was selected at Seattle, Washington, August, 1914, and the plants recorded.

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[1] In questions of nomenclature Piper's Flora of the State of Washington (19) has been followed

Herbs: Agrostis depressa, Athyrium cyclosorum, Cardamine pennsylvanica, Cardamine oligosperma, Cinna latifolia, Circaea pacifica, Claytonia sibirica, Epilobium halleanum, Epilobium adenocaulon, Equisetum telmateia, Geum macrophyllum, Leptaxis menziesii, Lysichiton camtschatcense, Polystichum munitum, Ranunculus bongardi, Ranunculus bongardi greenei, Roripa curvisiliqua, R. nasturtium, Stachys ciliata, Tiarella trifoliata, Washingtonia brevipes.

Shrubs: Echinopanax horridum, Ribes bracteosum, R. divaricatum, Sambucus callicarpa.

Trees: Acer macrophyllum, Alnus oregona.

The steep slopes of the ravine were covered with the following conifers: *Pseudotsuga taxifolia, Thuja plicata, Tsuga heterophylla* and *Taxus brevifolia.*

The later stages in the history of the river, *e. g.*, the establishing of the flood plain make possible a development of Lysichiton unattained in any of the previous stages. As the river grows old and the flow of the water sluggish the deposition of sand and silt becomes more effective. In the extensive mud flats formed in this way along or at the mouth of slow flowing streams, we often find Lysichiton more gigantic in size than anywhere else. The following data have reference to an alluvion of such nature located on the Little Spokane River at Waikiki (May, 1913). The ligneous vegetation was made up of *Alnus tenuifolia, Ribes aureum, R. irriguum, Salix lasiandra caudata, Salix sitchensis, Sambucus glauca, Spiraea menziesii.* The herbaceous vegetation was almost exclusively composed of *Lysichiton camtschatcense.* The mud lay bare between the thickets, and scattered here and there were *Limnorchis stricta* and *Cardamine pennsylvanica.* Typical estival plants were absent, the shade being too dense.

Likewise where wet meadows instead of forest cover the flood plains as is often the case in the Puget Sound region the conditions are very favorable for a luxuriant growth of Lysichiton. The extensive meadows around Bothell mainly composed of *Agrostis depressa,* *Alopecurus geniculatus fulvus*, *Poa pratensis* and *Panicularia pauciflora*, have an abundance of Lysichiton.

In order to make clear the relation of Lysichiton to the pond and swamp vegetation we have to outline briefly the development and succession of the plant societies within the pond-swamp series. The only justification in dealing with this topic so often discussed lies in the fact that no data on the subject have appeared with reference to Pacific coast conditions.

It will be convenient to group the different types of vegetation belonging to the pond and swamp series under two heads, which we for want of better terminology designate as half-drained and undrained ponds and swamps. Both types culminate in the coniferous climax forest, the former indirectly through a deciduous alnetum-salicetum, the latter without the intervention of a deciduous forest stage but through a typical sphagnetum. We find both types well represented in the Puget Sound region. As an example of the first type we may take Union Bay in Lake Washington. The well-known and much-discussed filling up process is going on here with *Nymphaea polysepala*, *Ceratophyllum demersum* and *Elodea canadensis* as the principal soil formers among the aquatic species. Further inland follows the cat-tail Dulichium associations (Transeau 25) with *Dulichium arundinaceum*, *Scirpus occidentalis*, *Sparganium eurycarpum*, and *Typha latifolia*. Back of this association and pushing forward often succeeding in reaching beyond it in places where the water is not too deep we have the two typical marginal plants *Polygonum amphibium* and *Potentilla palustris* fighting for supremacy and overrunning the somewhat earlier established, not seldom pure associations of *Menyanthes trifoliata* and *Hippurus vulgaris*. Next comes the shrub zone with *Salix cordata*, *S. geyeriana*, *S. sitchensis*, *Spiraea douglasii* and *Myrica gale*. There is often standing water between the thickets and pure associations of *Myosotis laxa* and *Oenanthe sarmentosa*. Encroaching upon this shrub zone we have the deciduous forest, almost exclusively made up of *Alnus oregona*. *Salix lasiandra* occurs in spots, and the characteristic shrub stratum is composed of *Cornus occidentalis*, *Lonicera involucrata*, *Rubus spectabilis* and *Sambucus callicarpa*. Scattered trees of *Thuja plicata* give evidence of the advance of the coniferous climax forest on the deciduous forest.

Lysichiton camtschatcense plays a prominent part in some of the successions outlined above, particularly in the alnetum and in the shrub zone. In the former situation it reaches the same gigantic size as in the forested alluvial deposits. Figure 2 shows the spring aspect of an alnetum where some windfalls have occurred (Lake Washington, April, 1914). *Lysichiton camtschatcense* grows here in an *Oenanthe sarmentosa* association with *Agrostis depressa*, *Angelica genuflexa*, *Epilobium adenocaulon* and *Myosotis laxa* as secondary species. A slow-flowing brook empties its water on the northern side and here (not visible in the figure) has a quite different flora established itself. *Roripa nasturtium*, characteristic of such localities, forms an almost closed carpet in which *Lysichiton camtschatcense*, *Agrostis depressa*, *Mimulus moschatus*, *Panicularia nervata* and *Veronica americana* are interspersed.

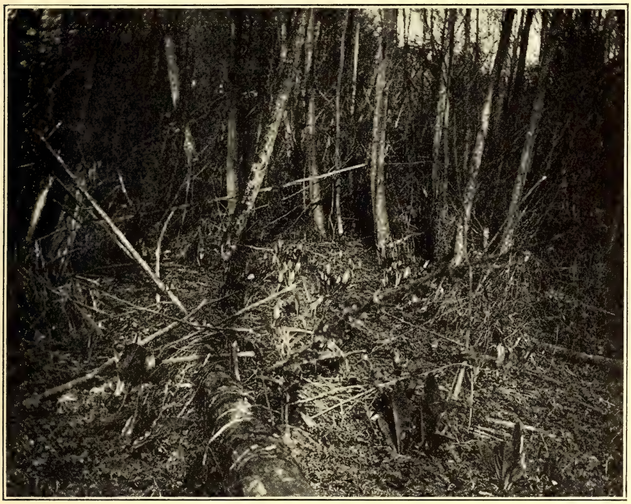

FIG. 2. Alnetum with *Lysichiton* and *Oenanthe sarmentosa*. Author's photograph.

When the topographic conditions are such that complete loss of drainage ensues a series of successions originate which differ radically from those of the half-drained swamp. Bog successions take the place of the former marginal successions and typical peat bogs are built up. In the Puget Sound region where a young topography and a moist climate favor the development of peat bogs, two different types can be distinguished, the second type deviating from the first one in so far as the sedge stage has been eliminated. In other respects the parallelism between the two types is perfect. Both arise and develop from an initial hydrophilous vegetation, both reach the fatal stage inaugurated by the ingress of Sphagnum and, finally, both culminate in the coniferous climax forest.

For an illustration of the first type we may take one of the numerous bogs on Mount Constitution in the San Juan Islands. This particular one is situated in a pocket in the mountain and is about 8 hectares in extent. There is open water in the central parts and a hydrophilous vegetation composed of *Menyanthes trifoliata*, *Nymphaea polysepala*, *Potentilla palustris* and *Chara*. Encroaching upon this vegetation and occupying most of the peripheral parts of the bog we have a typical sedge bog meadow (Dachnowski 8, 9), the low moor, grass moor or sedge moor of Warming (28) mainly composed of Carex. The ground stratum is made up of *Hypericum anagalloides* and mosses, notably *Hypnum giganteum*. The field stratum has the following herbs in addition to the Carex species: *Galium triflorum*, *Mentha canadensis*, *Menyanthes trifoliata*, *Potentilla palustris*, *Scutellaria galericulata*, *Veronica scutellata*, *Viola palustris* and in spots *Lysichiton camtschatcense*. A thick soft mass of Sphagnum occupies a small area in the middle of the bog bounding the open water vegetation. This supports the typical sphagnophilous plants of the region: *Ledum groenlandicum*, *Kalmia glauca*, *Oxycoccus oxycoccus intermedius* and *Drosera rotundifolia*. In places where the sphagnum has been killed, for reasons to be discussed later on, *Philonotis fontana* has stepped in and maintains the same characteristic plants as sphagnum including *Drosera rotundifolia*. That the sphagnum is spreading and will ultimately supplant the sedge vegetation is evident from the fact that small colonies of it have succeeded in establishing themselves among the sedges in advance of the main mass. Seedlings of *Pinus monticola* and *Thuja plicata* are common among the sedges, and dead trunks of *Tsuga heterophylla*, discussed more in detail below, are frequently met with.

The factors that condition the development of the second type

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[1] I am indebted to Professor T. C. Frye for the determination of the mosses.

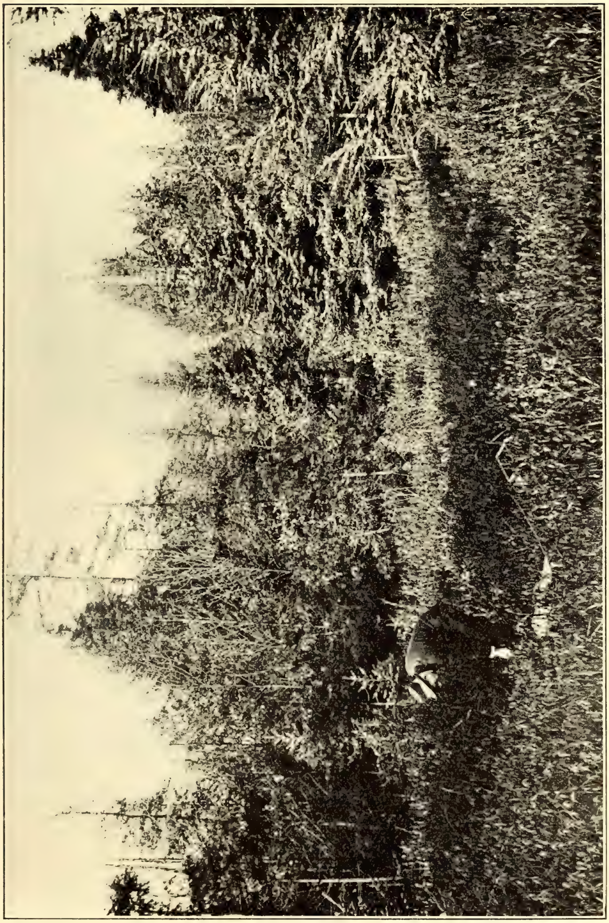

Fig. 3. A corner of Green Cake Bog. Photograph by Y. Westberg.

of peat bogs in which the sedge stage is omitted are the same as those of the first type, and they may all be referred to the lack of drainage. Just why the sedge stage is omitted is not altogether clear. The best example of the type in this region is that of Mud Lake. This lake was once connected with Lake Washington but has in geologically recent times been separated from it by a sand bar thrown up by wind and wave action. Extensive sphagnum bogs occupy the upper end of the lake. These were very likely initiated while Mud Lake still was a bay of Lake Washington. The building up of sphagnum bogs on floating mat vegetation can easily be followed in the southern portion of the lake. *Brasenia schreberi*, *Nymphaea polysepala*, *Potamogeton lonchites*, *P. Nuttallii*, *Utricularia vulgaris* and *U. minor* are very important in shallowing the lake. *Menyanthes trifoliata* is here as in other places (Cooper, 4, MacMillan, 16) the important mat-former by virtue of its mass of intertwined rhizomes. *Potentilla palustris* invades the association, spreads, reaches the edges of the mat and advances out in the open water. Fontinalis, aquatic Hypnum species and other mosses solidify the mat, which next is invaded by *Betula glandulosa* and an undetermined Salix species. Sometimes also *Spiraea douglasii* appears in this stage and often but not always *Typha latifolia*. This is the typical bog shrub stage (Dachnowski, 7, 8). The bog heath stage (Dachnowski, 7, 8) is initiated with the coming in of Sphagnum, which soon smothers the existing vegetation. The former shrubs are crowded out by *Ledum groenlandicum* and *Kalmia glauca* which together with *Oxycoccus oxycoccus intermedius* and *Drosera rotundifolia* establish themselves on the sphagnous substratum. As the xerophytic conditions increase resulting in the drying up of the sphagnum bog in the middle and older parts there is again brought about a change in the composition of the flora. Ledum becomes stunted, disappears entirely from the central parts but persists in the edges bounding the open water. The same is in somewhat less degree true of Kalmia. Cladonia species and *Eriophorum chamissonis* replace them. The bog forest (Dachnowski, 7, 8), is advancing with scattered *Tsuga heterophylla* and seedlings of *Pinus monticola* as forerunners.

The type of bog just described is by far the most common met with in the Puget Sound region. Indeed, the first type, distinguished by the sedge bog stage, is but poorly represented on the mainland. There is indication of such a stage in some places in Mud Lake and also in Spanaway Lake, near Tacoma; but neither is typical of the bog so well represented on Mount Constitution in the San Juan Islands. It might be a matter of difference in age between the bogs on the mainland and those of San Juan Islands, the former having advanced further than the latter and obliterated the sedge bog stage, but there are grounds for the belief that climatic factors are responsible for the dissimilarity.

The San Juan Islands, situated between the Strait of Juan de Fuca and the Strait of Georgia in the northern part of Puget Sound, have a lesser rainfall than most places on the mainland, probably because of the fact that they lie in lee of the Olympic Mountains (Piper, 19). The result is a difference in the flora of the two regions which in some respects is quite remarkable (Piper, 19, Turesson 27).

Many plants characteristic of the drier eastern parts of Washington occur on the islands while they are absent from the intermediate more humid regions. The similarity between the swamp flora of the San Juan Islands and the arid parts of eastern Washington is also great. Indeed, the composition of the swamp vegetation around Sportsman Lake on San Juan Island is almost identical with that of Newman Lake and Liberty Lake of Spokane County in eastern Washington. Both regions are too arid for Sphagnum. It is true, Sphagnum occurs in the northeast corner of Sportsman Lake, but it is not peat forming and cannot exist outside of the protective zone of willow thickets. The southern end of the lake has an extensive association of *Ledum groenlandicum* but Sphagnum is absent. Similarly, the sedge bog meadow on Mount Constitution has great resemblance to the sedge bog meadows in eastern Washington and it is only the slightly higher altitude, and as a result the somewhat greater humidity that makes peat formation through Sphagnum possible on the former locality. However, on the mainland where a more humid climate favors the growth of Sphagnum, the development of the bog becomes altered due to the early ingress of the white moss. The edaphic conditions for a luxuriant growth of Sphagnum are fullfilled already before the establishing of the floating mat vegetation. The interpolation of the floating mat stage of a purely hydrophilous vegetation cannot in itself represent a step towards more xerophytic conditions favoring the growth of Sphagnum and the sphagnophilous flora. These are, as stated above, already in evidence before the floating mat stage has been reached, and the mat merely serves as mechanical support for the Sphagnum and its associates. In Lake Kapowsin, near Tacoma, logs floating in the water have taken the place of the floating mat, and *Drosera rotundifolia*, *Kalmia glauca* and other bog xerophytes flourish in the thin layer of humus covering the logs.[1]

When the rolling sphagnum cover has reached out to the open water bounding the floating mat it plunges down in the water by continual growth from behind thus filling in the pond. This may be seen in many places, for instance, at Mud Lake, Echo Lake and Crystal Lake (compare also Dachnowski, 7, Eriksson, 10). In the latter locality it also succeeds in building extensive mats out into the lake supported by the branches of *Ledum groenlandicum* (compare here Gates, 11).

Some additional facts may be brought out with regard to the various stages in the peat bog vegetation of the region. It is clear that smaller deviations may occur from the development of the two types described in the above. The bog shrub stage, which in this region precedes the bog heath stage, may be wanting or but poorly developed. It is represented by *Spiraea douglasii* in a bog near Maltby. There is also some difference in the composition of the bog forest. The relations of this stage to the climax forest is somewhat difficult to determine as the actual nature and composition of the climax forest is still a matter of conjecture. The demineralization of forest soil due to the humid climate, and the rapid accumulation of humus over the mineral soil layer favor the growth of *Tsuga heterophylla* and *Thuja plicata* while it hinders the development of *Pseudotsuga taxifolia* which thrives best on mineral soils. Undoubtedly, Tsuga and Thuja would together dominate the lowland region of the mainland were it not for the extensive forest fires which by destroying the humus mantle prepare the soil for Pseudotsuga. The belief that the latter tree is excluded from the culminating type of forest vegetation is further strengthened by the fact that Pseudotsuga is unable to seed under its own shade while seedlings and young trees of Tsuga and Thuja are common in the mature Pseudotsuga forest.

With these points in mind the question of the relation of the bog forest to the climax forest, here supposed to be composed of Tsuga and Thuja, will become clearer. The conifers making up the bog forest on the various peat bogs of the region are the following: *Picea sitchensis*, *Pinus contorta*, *Pinus monticola*, *Thuja plicata*, *Pseudotsuga taxifolia* and *Tsuga heterophylla*. Only three of them are important, namely: *Pinus contorta*, *Thuja plicata* and *Pseudotsuga taxifolia*. *Picea sitchensis* and *Pinus monticola* have a very limited distribution in this region. Their occurrence in peat bogs is probably due to lessened competition in the latter localities enabling these species to persist in a region not otherwise favorable to their development. The occurrence of Pseudotsuga in the Green Lake bog, discussed in detail below, is likely to be attributed to the partial drainage of that bog, Rigg (21). Of the remaining, *Pinus contorta* and *Tsuga heterophylla* are especially worthy of notice. Tsuga is always confined to the youngest and wettest peat bogs, in fact, it is the first conifer to appear when the Sphagnum has become firm enough to support trees. When the sphagnum bog gets older and drier Tsuga is killed. The many dried up trunks of Tsuga in older peat bogs or in older and drier parts of a peat bog are remnants from a younger and wetter stage in the history of the bog. The bog forest of the sphagnum bog in its dried stage is a pure growth of *Pinus contorta*. This tree is confined to dry hills and gravelly prairies outside of peat bogs. The occurrence of a plant both on peat soils and on dry, sandy soil has repeatedly been observed in America as well as in Europe and Dachnowski (9) has recently added new examples to the list.

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[1] *Rigg* (22) calls attention to the noteworthy fact that *Drosera rotundifolia*, one of the most typical sphagnophilous plants, occurs outside of the sphagnous substratum in this locality. Its occurrence on a *Philomatis fontana* carpet on Mount Constitution, already referred to, becomes of interest in this connection.

It follows that the relative age of the bog can be determined from the nature of the bog forest. The bogs of Mud Lake with Tsuga and the Crystal Lake bog with *Pinus contorta* are illustrative examples.

The position of *Thuja plicata* with regard to its resistance to dryness seems to be between that of Tsuga and *Pinus contorta*. In eastern Washington and in the Puget Sound region it occurs on dry xerophytic slopes as well as in swamps thus showing the same indifference to habitat as in the Chicago region (Cowles, 6) and approaching in nature *Pinus contorta*. It is not common in the peat bogs of the region (Mount Constitution), but occurs in bogs partially drained.

It is through the two above mentioned stages in the bog forest, the Tsuga association and the Pinus contorta association, that the climax forest will be reached. More extensive investigations have to be undertaken before the course of development after the pine association can be ascertained. As the xerophytic conditions decrease a forest composed of Tsuga and Thuja will in all likelihood establish itself and conclude the series of successions.

Having given a brief account of the nature and character of swamp and bog vegetation in this region we will now discuss in fuller detail the behavior of *Lysichiton camtschatcense* in sphagnum bogs. The bog in which the following observations have been made is that of Green Lake lying within the city limits of Seattle. Most of this bog, formerly about 12 hectares in extent, has been drained and turned into arable soil and only about 0.2 hectare now remain bearing the original bog vegetation. Rigg (21) has in his study of the toxic properties of waters from different sphagnum bogs of the region given a brief sketch of the flora of this bog. From the fact that the water from the bog had no toxic effect on the development of root-hairs in Tradescantia, probably on account of partial drainage, he concludes that plants not found in typical bogs had been able to enter and establish themselves. The flora is doubtless somewhat mixed in character. Figure 3 shows a part of the bog. *Ledum groenlandicum* dominates the area with Kalmia, Oxycoccus, and *Drosera rotundifolia* as secondary species. So far the flora is that of the typical sphagnum bog. However, in addition to the trees commonly found in peat bogs, namely *Tsuga heterophylla* and, in rarer cases, *Thuja plicata*, *Picea sitchensis* and *Pinus monticola*, we find *Pseudotsuga taxifolia* and *Alnus oregona*, and, among shrubs, *Salix scouleri* and *Cornus occidentalis*. The occurrence of these latter might be explained on the basis of partial drainage of the bog. The occurrence of Lysichiton in the bog requires, however, another interpretation. There is, furthermore, another peculiarity which in a still higher degree than the mere presence of Lysichiton demands an explanation. Holes of considerable depth and width are scattered all over the area. Lysichiton is strictly confined to these holes. Firmly rooted in the bottom it occupies most of the space by virtue of its large and numerous leaves which only partly reach out of the hole. In the following table measurements of a number of representative pits are given in centimeters, the width being that of the upper edge.

The form of these holes is mostly circular and wider above than below. Figure 4 is a photograph of one of them in winter-time. Remains of last year's skunk cabbage leaves are seen at the left. The characteristic vegetation of Ledum, Kalmia and Oxycoccus surrounding the pit can also be seen. A further fact to be born in mind is that the hole sunk in the soft mass of Sphagnum has its sides covered not with a growth of Sphagnum but with a number of other mosses and, occasionally, of Cladonia and Peltigera species. The following mosses are found to be common in the upper more exposed parts of the holes: *Dicranum scoparium*, *Hylocomium triquetrum*, *Hypnum cuspidatum* and *Hypnum splendens*. The sides farther down are covered with *Aulacomnium androgynum*, *Brachythecium asperrimum*, *Eurhynchium strigosum* and *Mnium punctatum*.

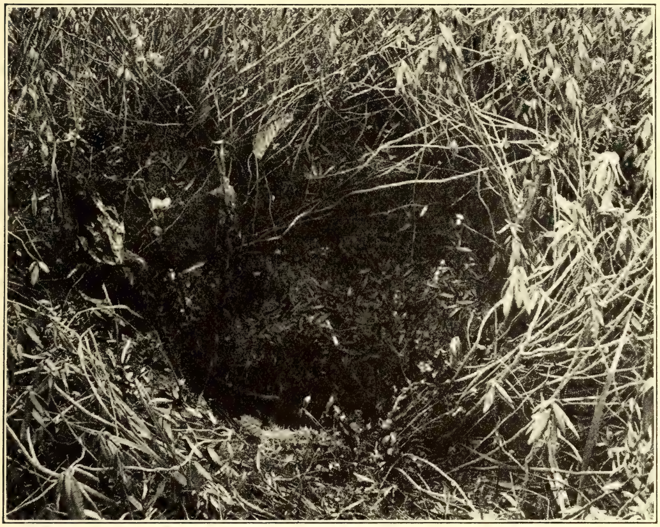

FIG. 4. A skunk cabbage pit, winter time. Photograph by Y. Westberg.

How do these holes originate? Considering the great bulk of leaves produced by Lysichiton in course of the summer and bearing in mind that they rot in winter-time the assumption lies near at hand that the skunk cabbage kills the surrounding Sphagnum. If so, the question as to whether the leaves cause the death of the Sphagnum by the contact itself or indirectly by shading becomes of interest.

In the spring, 1914, a square 1 meter by 1.5 meters in size was laid out in the middle of the bog and decaying leaves of Lysichiton were spread in one-centimeter-thick layer over the selected square.

Another square of the same size was selected and covered with decaying leaves of *Alnus oregona* and *Cornus occidentalis*. When uncovered and examined in the fall the Sphagnum was blackened and apparently killed in both squares. Tufts of this material were brought to the laboratory, put in test tubes partly filled with water and allowed to remain for a month, the water being changed every week. Some of it was put in moist chambers. The moss did not recuperate in either case. Sphagnum planted in test tubes partly filled with water gotten by squeezing decaying Lysichiton leaves' grew well. Also when planted in soil collected from the bottom of the skunk cabbage holes Sphagnum showed considerable growth.

The killing of Sphagnum by the skunk cabbage leaves was first thought as being the result of some toxic substance in the skunk cabbage. With the investigation of Baumann and Gully in mind supplemented with those of Skene (24) on the injurious effect of alkalies on the growth of Sphagnum, a number of samples of decaying skunk cabbage leaves were tested. They all gave acid reaction to litmus and phenolphthalein. Any interference with the absorption of mineral salts due to the neutralization of the acid colloids in the hyaline cells of Sphagnum or of the acid medium is therefore out of question. As the leaves of Alnus and Cornus also destroyed the Sphagnum the phenomenon cannot be explained by assuming the presence of a specific toxic substance in the skunk cabbage leaves. The property must be one common to both Lysichiton and Alnus-Cornus leaves.

The cause most likely responsible for the killing of the Sphagnum and which adequately explains both cases is the one of shade and mechanical injury produced by the mass of overlying leaves. That Sphagna are light-demanding plants and very sensitive to waste are well known facts pointed out by almost every worker in the field. Transeau (26) makes the statement that in the Huron River Valley Cassandra affords a very suitable framework for the upbuilding of Sphagnum, among other reasons because its shade does not interfere with the photosynthetic work of the moss. Gates (11) points out that in the bog he investigated the white moss was confined to the margin of the lake "where it is not shaded by grass or bushes." Also Haglund (13) makes the statement that Sphagna are light-demanding plants. As regards their sensitiveness to waste, numerous instances are reported in the literature. Dachnowski (7) notices that covering of fallen foliage suppresses the growth of Sphagnum, Oxycoccus and similar plants. The following is a quotation from Cooper (4): "Because of the shade which Ledum produces and the considerable amount of waste which falls from it, the upward growth of the moss is gradually retarded and finally ceases altogether. About this time or often before, young plants of other mosses more or less tolerant of shade become established upon the higher parts of the Sphagnum moss. *Polytrichum strictum*, *Aulacomnium palustre* and *Calliergon schreberi* soon follow. These species form mats of continually increasing lateral extent which put an effectual stop to further upward growth of Sphagnum." The same facts are brought out in another paper by this author (Cooper, 5). Similarly, Raunkiaer (20) calls attention to the harmful influence of the shady spruce forest on the Sphagnum bog as well as to the injurious effect of leaf fall on Sphagnum. The best illustration of the sensitiveness of Sphagnum to shade is given by Eriksson (10) in his studies on the peat bogs in the middle part of Sweden. It may be cited here. If a spruce has succeeded in establishing itself on top of a Sphagnum hummock in the bog it will ultimately be killed. As Sphagnum is unable to grow in the dense shade below the spruce a depression is formed here. This depression becomes filled with water which results in the death of the tree. Similar "Beschattungs-Schlenken" have prior to Eriksson been described and explained by Sernander (vide Eriksson).

When viewed in the light of these additional data it is easy to see how the deep skunk cabbage pits in the Green Lake bog originated. Sphagnum is unable to grow in the dense shade cast by the large skunk cabbage leaves during the vegetative season. In late fall when the leaves rot, an area all around the plant becomes covered with a thick layer of decaying leaves which smothers and mechanically injures the Sphagnum if it has succeeded in encroaching upon the area dominated by the skunk cabbage. In this way the Sphagnum is kept at a distance, and the pit deepens as the Sphagnum layer continually thickens in the edges not in reach by the skunk cabbage. Finally mosses more tolerant of the deep shade and waste gain entrance and form mats on top of the Sphagnum in the way described by Cooper.

A few additional instances from this region of the sensitiveness of Sphagnum to waste and shade may be cited. It was stated above that Sphagnum had been killed in certain places of the Mount Constitution bog and replaced by *Philonotis fontana*. This was particularly noticeable in the vicinity of Ledum thickets where the shade was dense and waste abundant. An observation somewhat similar to that of Eriksson can be made in the Green Lake bog. Slight depressions are found beneath the hemlocks, the Sphagnum being unable to grow here on account of the shade. The trees are not killed, however, as Tsuga is able to endure very wet soils.

The origin of the marginal ditch surrounding sphagnum bogs in some regions has been discussed from time to time. In order to reach a satisfactory explanation we have to consider not only the factor of shade as expounded by Cooper (4) and Atkinson (1), but also the factor of waste. Fallen leaves and other organic material are swept from the forest floor into the edge of the pool and tend to smother the vegetation (Shaw 23). The combined factor of shade and waste is very likely responsible for the marginal ditch, although suggestions of another nature are made by some. (Burns 3, Gräbner in Warming 28).

It has been shown how Lysichiton is able to hold its own in the sphagnum bog and how as a consequence deep pits are formed. In order to determine more definitely the relation of Sphagnum to Lysichiton and if possible to ascertain in which stage and at what time in the history of the peat bog Lysichiton made its entrance, a cross-section was made through one of the skunk cabbage pits extending one meter beyond the pit and two meters down through the peat. The analyses of the samples of peat brought up from this ditch were supplemented with those made from a ditch already dug in the peat bog for the purpose of draining the bog. To complete the data on the stratification of the bog, test borings were made from the bottom of these ditches. Figure 5 shows the nature of the stratification of this peat deposit.

The area now occupied by the peat deposits is evidently an old lake basin on a bed of drift clay. The outlet of the former lake was obstructed relatively early and the course of development followed that of the undrained swamp. This is confirmed by the entire absence of remains of alder, deciduous shrubs and other plants of the half-drained swamp. That the development has gone through the bog sedge meadow, poorly represented on the mainland at present, is seen from the thick layer of Carex peat overlying the clay. From a wet stage the bog reached a drier stage and big trees grew up. The large stool *in situ* and the remains of *Gaultheria shallon* bear witness of this relatively dry stage. The replacement of the forest by the Sphagnum denotes the critical stage in the history of the bog. The exact cause of the destruction of the forest is very difficult to conjecture. Changes in the level of the ground water is a reasonably safe assumption. Some of the current opinions on this topic will be discussed below. For our interpretation of the past history of Lysichiton in

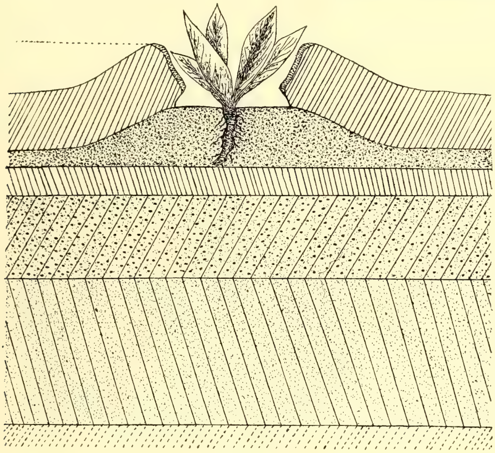

FIG. 5.

A. 60 cm. of *Sphagnum teres* peat containing leaves and root systems of the present flora, notably of Ledum, Kalmia and Oxycoccus.

B. 15 cm. of brownish black mud.

C. 20 cm. of *Sphagnum palustre* peat containing an abundance of leaves and twigs of Oxycoccus, rarer leaves of *Tsuga heterophylla*, *Kalmia glauca*, grasses, and twigs of *Thuja plicata*.

D. 90 cm. of forest peat consisting of wood detritus and bark mixed with leaves, twigs and cones of *Thuja plicata*, leaves and twigs of *Tsuga heterophylla*, leaves of *Ledum groenlandicum*, *Kalmia glauca*, *Gaultheria shallon*, grasses, seedwings of *Pinus monticola*, and whole plants of *Camptothecium sylvaticum*. Coal was occasionally met with. One large stool *in situ* was found.

E. 150 cm. of Carex peat highly mouldered below with leaves of Kalmia and Ledum, and an abundance of *Hypnum giganteum*, and sparse Sphagnum.

F. Clay.

G. Moss peat.

the bog the layer *B* is, however, of great interest. This amorphous deposit of a brownish black color has been laid down in water. By some cause or another the bog was flooded and a rich vegetation ensued which gave rise to the mud layer in question. The history of Lysichiton in the bog goes back to the time of the depositing of this layer. When the water subsided, probably while water still remained in the depression during the wet season, Lysichiton established itself. From our acquaintance with its habitat in ravine pools where such mud arises from the decomposition of leaves and other organic material swept into the pool, and through our familiarity with its abundance on alluvial mud banks, we perceive what a congenial habitat such a deposit would afford. It is seen from the cross section that Lysichiton is rooted in this mud layer. The annual decay of great masses of leaves has added to the layer and an extensive mud pad has been built up on top of the original deposit. The second white moss invasion followed and the soft mass of Sphagnum rolled out over the ground occupied by the swamp flora burying and suppressing it with the exception of the skunk cabbage which was able to hold its own against the oncoming white moss by virtue of mere bulkiness. The factors of shade and waste in the formation of the skunk cabbage pits have been discussed above, and the figure makes clear the structure of these pits. During the rainy reason, fall and winter, water collects in the pits, and erosive action loosens the Sphagnum layer from the mud pad resulting in a concavity between the two strata.

Thus the conclusion is arrived at that Lysichiton in the Green Lake bog is a relict from the swamp flora and not related to the group of plants which in recent times have succeeded in entering the bog by reason of partial drainage.

As to the devastation of the forest by Sphagnum indicated in the deposit by the Sphagnum stratum (*C*) overlying the forest bed (*D*), we doubtless evade the difficulties rather than explain them by assuming an alternation of moist and dry periods of great length corresponding to the alternation of strata of Sphagnum and forest in the peat bogs as was done by Geikie (12) and Blytt (2) and after them by Scottish and Scandinavian botanists in particular. The conversion of forest into marsh can take place and be explained without resorting to such bold conjectures. Nilsson's (17) account of the present swamping of the forests in Sweden by Sphagnum is a case in point. Haglund (13) has in a most interesting and convincing way demonstrated that the stools of the forest beds in most of the Swedish peat bogs have been on fire. By burning, the peat became unsuitable for the growth of forest vegetation, while on the other hand Sphagnum found a very favorable habitat and replaced the forest. Furthermore, with the disappearance of the forests an elevation of the level of the ground water takes place and hydrophytic conditions increase, resulting in a corresponding change in vegetation. Burns (3) gives an interesting account of the effect of alternating dry and wet periods of short duration on bog vegetation in the Huron River Valley. His description of the alternating layers of Sphagnum and Polytrichum peat corresponding to the wet and dry periods of previous years is particularly illustrative.

Examples of the destruction of forests under the influence of increased humidity due to local conditions can also be cited from this region. Lake Kapowsin, near Tacoma, originated by the damming up of a river probably by beaver dams and by subsequent swamp vegetation. The surrounding forest was flooded, and the trunks sticking up out of the water bear witness of the flood. Today floating bog vegetation occupies part of the lake's surface. Examples of a somewhat similar nature have been described repeatedly (for instance by Nilsson, 17). The above-mentioned sedge bog meadow on Mount Constitution may also be taken as an example. It occupies a deep pocket in the mountain. In the transition zone between the bog and the adjoining forest almost every tree is killed. The fringe of dead trees is as marked as if cut out with a knife. As the swamp vegetation increased and filled up the concavity it reached the forest which had established itself on the slopes with the result that this was killed either by the water itself or by toxic substances in the stagnant water.

In conclusion I want to call attention to the different ways in which plants enter and have entered the Green Lake bog. In addition to Lysichiton there occurs in the bog an undetermined Carex species which probably also is a relict from the swamp stage. It is usually sterile and illustrates the tendency of plants to become sterile or xerophytic in structure when growing under bog conditions as shown by Lindman (15) and Nilsson (18) for a number of plants and confirmed by the cultural experiments of Transeau (26) and Dachnowski (9).

A point fully as interesting as the relicts in the bog is furnished by the flora that occupies the bottom of the skunk cabbage pits. The nature of the flora which finds shelter here will become clear from the following list of plants found in six different pits.

I. *Angelica genuflexa, Limnorchis leucostachys robusta, Viola palustris.*
II. *Angelica, Galium aparine, Trientalis latifolia.*
III. *Angelica, Athyrium cyclosorum, Gaultheria shallon, Spiraea douglasii.*
IV. *Alnus oregona* (seedling) Angelica, Athyrium, *Gaultheria shallon, Hieracium scouleri, Lonicera involucrata, Rhamnus purshiana, Sambucus callicarpa* (seedling), *Spiraea douglasii* (seedling).
V. *Athyrium, Carex* sp.
VI. *Epilobium adenocaulon, Carex* sp. *Potentilla palustris.*

Most of the above mentioned plants do not produce any flowers on account of deep shade and crowded conditions in the pits. However, seedlings of trees often get a start here and some succeed in reaching further development. Many of the trees and shrubs now growing in the bog, as for instance *Alnus oregona* and *Cornus occidentalis*, began in all likelihood their development in one of these pits.

The importance of the skunk cabbage pits and their floras in the replacement of the xerophytic bog flora by a more mesophytic is perhaps greater than it appears to be. There is no doubt, however, that partial drainage of the bog also has played its part in bringing about the present composition of the vegetation.

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SIGNIFICANT ACCURACY IN RECORDING GENETIC DATA

E. M. EAST

In 1913, I contributed a paper to the Botanical Gazette (55: 177–188) on the inheritance of flower size in a cross between *Nicotiana alata grandiflora* Comes, and a type thought to be *Nicotiana forgetiana* Hort. Sand. Corolla size had been selected for study because in this genus it is "so comparatively constant under all conditions attending development"—something which could not be said of any other size character that had been under observation. Since other investigations of the same kind were under way, and a larger amount of data might be reported later, the "liberty of asserting the truth of this statement" with only the following data in its support was requested. This paragraph followed.

"During the past four years, I have grown about 20 species of Nicotiana in considerable numbers. They have been grown under very diverse conditions. Some have been starved in four-inch pots, others have had the best of greenhouse treatment; some have had poor field conditions, others have had all field conditions practically at their best. The height of the plants, the size of the leaves, and similar size complexes have varied enormously, but the size of the corollas has scarcely varied at all. For example, plants of *Nicotiana sylvestris* Speg. & Comes, grown to maturity in four-inch pots, produced no leaves longer than 7 inches. On the other hand, sister plants of the same pure line produced leaves 30 inches long in the field. Both series, however, produced flowers with the same length and spread of corolla. Furthermore, cuttings from 20 of the field plants reported in this study were rooted and grown in small pots (6 inch) in the greenhouse. Their blossoms were the same size as those of the field grown plants from which they came."

[The **Journal** for April (3: 135–210) was issued April 18, 1916.]

Recently Goodspeed and Clausen have published in this Journal (**2**: 332–374. 1915) an immense amount of data on the influence that certain environmental factors have on flower size in Nicotiana. The conclusions they draw are eight in number based upon 25,000 measurements of the length and spread of the corollas of *Nicotiana tabacum* var. *macrophylla* and three hybrids between *N. tabacum* varieties and *N. sylvestris*, and run somewhat as follows:

1. Both length and spread of corolla decrease during the flowering season to such an extent that at the end of six weeks the average spread may drop 6 mm. and the average length 4.5 mm.

2. The F_1 *N. tabacum* × *N. sylvestris* hybrids are short-lived perennials, and the flowers of the second season are of approximately the same size as those of the first season.

3. Removal of open flowers during the normal flowering season prevents nearly all decrease in size.

4. Flowers apparently fully opened are smaller before than they are after anthesis, even though the anthers are partially sterile.

5. Flowers on pot-grown cuttings are smaller than those borne on the field plants from which they were taken.

6. Under favorable and unfavorable greenhouse conditions, flower size varies distinctly and in the same direction as the vegetative characters.

7. Length of corolla is more stable than spread of corolla under environmental stimuli.

8. "The only true distribution representing the flower size of a population must be based upon measurements which, for each plant, extend over the greater part of the period of flowering normal for the given species or hybrid group, or cover an identical portion of the flowering period of each plant."

The data were collected and these conclusions drawn, the authors say, "to establish tentative criteria in keeping with which flower size investigations, in Nicotiana at least, should be carried on and interpreted."

The statements of Goodspeed and Clausen and those quoted from my own paper seem at first sight to be irreconcilable. Indeed, the authors have done me the honor of devoting a considerable portion of their paper to criticizing my views and methods. For example, because it was maintained that flowers are constant under different environments compared with the changes exhibited by vegetative organs, they have assumed that no precautions whatever were taken to eliminate environmental differences. Since the statement was made that plants were grown under diverse conditions, a fact mentioned merely in connection with the question of the effect of stimuli on corolla size, they seem to have concluded unjustly and unreasonably that the data from *these* experiments were used in the paper under consideration.

On the other hand, Goodspeed and Clausen are perfectly justified in asking for a description of the way in which my data were taken. I wish to make such a statement, therefore, in order to support my former paper and some other studies on the inheritance of flower size which are to be published in the near future, and because of the opportunity presented to illustrate a question of considerable general interest. This question, which as a teacher of genetics I have found neglected by research students more than any other, is: *What is significant accuracy in recording data?*

The seemingly opposed statements of Goodspeed and Clausen and of myself serve to illustrate the thought in mind. The two allegations are not wholly discordant. Although I do not wish to withdraw or to modify my own statements, at the same time I am willing, in a broad sense, to accept most of their conclusions. Excluding certain differences in our data that are undoubtedly due to dissimilar conditions at Berkeley, California, and at Boston, Massachusetts, my own results are similar to theirs except as to the magnitude of the changes caused by environmental differences. The point upon which we differ decidedly is the *significance* of the results in relation to the problem at hand—the inheritance of differences in corolla size in Nicotiana.

One of my college instructors once said to me: "It is seldom necessary, in the interests of scientific accuracy, to weigh a ton of hay on an analytical balance." That statement might be made the basis of a course on Precision of Measurements. One is hardly ever required to impress mechanical accuracy upon really earnest students. They will weigh and measure material with the utmost pains (in spirit at least). What is difficult is to impress an idea of true precision. It is not uncommon to see measurements recorded to tenth millimeters after the random use of two scales having a one percent difference, or material for analysis weighed to the fourth decimal place with weights that have never visited the Bureau of Standards, on a balance with very unequal arms. It is rare to find students who think of these errors and endeavor to correct them, although such correction is as necessary in biology as in physics. Let us see how our biological problem fits the rules for the treatment of errors in use in experimental physics.

It was desired to record, in such a manner that they would be comparable, numerics that represented the phenotypes of series of plants of species of Nicotiana in regard to corolla length and spread, sufficiently accurately that genetic analysis of the results might be made.

The investigation was initiated by a series of preliminary measurements designed to show the practical physical limits to the precision of the direct measurements. Repeated measurements of the same flowers showed that there were residual errors beyond one millimeter in the case of length and two millimeters in the case of spread of corolla. Measurement to millimeters was adopted, therefore, although these measurements were afterwards thrown into larger classes for reasons that can be justified biometrically.

Then came a study of ontogenetic variation in order that the factors affecting such variation might be detected. The factors that would naturally occur to anyone who had had experience in growing plants were time of planting, physical and chemical condition of the soil, moisture, age of plant, flowering period, age of flower, position of inflorescence on plant and position of flower in the inflorescence. To determine the effect of each of these factors, it was necessary of course to eliminate the influence of all the others as far as possible. Since the cultures to be compared were nearly always planted at the same time, and since this variable is somewhat dependent upon others that were under consideration, it was neglected. My cultures have also been grown in well-drained soil very uniform in its fertility, but it was thought wise to determine how much effect extreme soil conditions might have. Several species growing outside in soil of good tilth were compared with greenhouse pot cultures. Three-inch, four-inch, five-inch and six-inch pots were used in various species, but the treatment was uniform for each species. The species were *N. tabacum* (several varieties), *N. rustica* (several varieties), *N. longiflora* (two varieties), *N. sylvestris*, *N. paniculata*, *N. acuminata*, *N. forgetiana* and *N. alata grandiflora*. Since only from ten to twenty plants could be grown in the greenhouse in most cases, statistical constants were not calculated, for I have not the faith of Goodspeed and Clausen in probable errors based on nine or ten observations (see their tables II *a*, *b* and III *a*, *b*). Averages of five flowers per plant taken when first in full flower, however, indicated means within a millimeter of each other for length and within two millimeters of each other for spread of corolla for over half of the species, when compared with the sister plants in the field. The greatest difference was in a *N. alata grandiflora* test where the starved plants showed an average of about 5 mm. shorter and 7 mm. narrower flowers. Hybrids were also tested. As I do not consider it necessary to cite figures endlessly where they serve so little purpose, however, only a table of results on a cross between two varieties of *N. longiflora* is given, the field records and the pot records being made by different observers. The general effect of starvation can be seen even without having the means calculated. A comparatively small number of observations were made on each population, but they serve as samples of the frequencies found. Certainly no marked decrease in size is apparent, and since the vegetative organs of the pot-grown plants varied from one half to one fifth the size of those in the field (linear dimensions), it seems that one

TABLE I

Frequency Distribution for Length of Corolla in Cross between N. longiflora Varieties

Designation	Class Centers in Millimeters																				
	37	40	43	46	49	52	55	58	61	64	67	70	73	76	79	82	85	83	91	94	97
No. 383 field..	4	32	1	...	...	...	...	...	...	...	...	...	...	...	...	...	.	.	.	...	.
No. 383 pots..	3	15	1	...	...	...	...	...	...	...	...	...	...	...	...	...	.	.	.	...	.
No. 330 field..	.	...	.	...	...	...	...	...	...	...	...	...	...	...	...	...	.	5	7	10	2
No. 330 pot...	.	...	.	...	...	...	...	...	...	...	...	...	...	...	...	...	.	1	4	3	1
(383 × 330) F_3A field....	.	...	.	...	...	...	...	...	...	4	20	25	59	41	19	2	.	.	.	...	.
Ditto, pot.....	.	...	.	...	...	...	...	...	...	...	1	3	4	1	...	...	.	.	.	...	.
(383 × 330) F_3B field....	.	...	6	20	53	49	15	4	...	...	...	...	...	...	...	...	.	.	.	...	.
Ditto, pot.....	.	...	.	2	6	4	...	...	...	...	...	...	...	...	...	...	.	.	.	...	.
(383 × 330) F_3C field....	.	...	2	3	9	25	37	70	19	10	...	...	...	...	...	...	.	.	.	...	.
Ditto, pot.....	.	...	.	...	...	2	3	3	...	...	...	...	...	...	...	...	.	.	.	...	.
(383 × 330) F_3D field ...	.	...	.	...	...	...	...	...	2	8	14	21	39	39	32	10	1	.	.	...	.
Ditto, pot.....	.	...	.	...	...	...	...	...	...	...	1	...	3	3	2	3	.	.	.	...	.
(383 × 330) F_3E field....	.	...	.	...	1	...	1	1	1	2	16	33	43	34	20	6	1	.	.	...	.
Ditto, pot.....	.	...	.	...	...	1	1	...	...	...	...	1	4	1	2	...	.	.	.	...	.

should be justified in stating that comparatively starvation had no effect on the flowers.

Both sets of these plants had a sufficient supply of moisture to keep them healthy. When this is not the case there is some difference in flower size. For example, some *N. rustica* plants each showing a mean flower length of 20 mm. with extremes of 18 mm. and 22 mm. at the first of the season, decreased in their mean flower length to 18.8 mm. after being in flower for four weeks during which very little rain fell. Then came four inches of rain within forty-eight hours. After this, stout vigorous laterals arose from the lower part of the main stems bearing flowers with a mean length of 21.1 mm. (extremes were 19 mm. and 23 mm.). Thus a marked difference in activity of cell division shows its effect on the flower.

This factor is probably the cause of the greater size shown by flowers on lateral branches when compared with those on terminal branches in Goodspeed's and Clausen's work (Tables XIII, XIV, XV). These authors also found that the flowers on new vigorous branches after "cutting back" were increased in the same way.

These facts should be taken into consideration when examining the conclusion of the California botanists that flower size decreases markedly as the length of the flowering season increases. Their data, as well as my own, proving that flower size may keep up to that of the first of the season and even increase if the weather conditions remain favorable for the production of vigorous new lateral branches, show that it is questionable whether a significant decrease in flower size occurs during the time that data would be likely to be taken. Their data showing marked decreases from the first of the season to mid-season are from populations of 9 and 10. During similar periods I have found no measurable decrease in flower length in *N. tabacum*, *N. longiflora*, *N. paniculata* and *N. rustica.* I have found a mean decrease of 1.0 mm. to 1.5 mm. which possibly is due to this factor in certain cultures of *N. langsdorffii*, *N. acuminata*, *N. forgetiana* and *N. alata grandiflora*, but I think the true occasion of the decrease was lack of moisture. On the other hand, there seems to be evidence in Goodspeed's and Clausen's data that toward the end of the season there is likely to be a decrease in flower size. My own data have shown a drop of from 4 mm. to 8 mm. in both corolla length and spread in various species in the last dozen or two flowers produced. This shows as a sudden change which is evidently due to physiological reasons. The true state of affairs is masked, therefore, when this decrease is treated as a gradual drop in flower size during the season. If measurements on greenhouse cultures grown in proper sized pots are taken daily over a long period, they simply show comparative uniformity in flower size until about the end of the flowering season. Then a decrease which produces a sharp bend in the curve occurs.

As to variation in size owing to age of the flower, I have found that this is largely a mechanical difficulty. There is no difference in length between flowers before and after anthesis, for anthesis takes place normally either before or within 10 hours after the flower opens in all species of Nicotiana under Boston conditions. A flower if unpollinated may open for as many as 5 successive days, and there is a slight increase in both length and spread of the corolla. But a pollinated flower seldom opens on more than two successive days. The flower becomes less firm however and the *spread* of the corolla may *appear* to increase.

Flowers of the same relative position on vigorous branches are the same size whether they be on the main stalk or on laterals in species like *N. forgetiana* and *N. alata grandiflora* which are characterized by vigorous lateral branches from the base of the stem. Flowers on lateral branches in species like *N. tabacum* where the main stem is so much more vigorous, average (in my counts) slightly less (under 1 mm.) than those on the main stem.

After about the sixth flower on the species having racemes, and on the flowers coming out after the first full glory of the panicled species, there is also a slight decrease in size owing to decrease in the conducting channels of the fibro-vascular system.

What information do these observations, which are the preliminary "qualitative" tests made in every investigation, give us? They show that to record the phenotypes of flower size of a series of Nicotiana plants, the seeds should be sown at the same time in uniform soil, the plants should be pricked out uniformly and set at the same time in a plot of uniform fertility. The flower records should be made within two or three weeks of each other at the first of the season, allowing no marked climatic change to intervene if possible. The flowers recorded should be the vigorous flowers (as stated in the last paragraph) of vigorous branches, and should be measured on the same day that they open.

This procedure should be followed where it is physically possible, and any departure noted in order that a correction for any constant error due to it may be calculated, if it be advisable. But, one might ask, would not any trained geneticist have taken these precautions anyway? What has been gained?

The advantages are real. Unsuspected constant errors often come to light through such preliminary investigations. The good fortune that none appeared here certainly makes it no less satisfactory. It showed that control of conditions in such a manner that constant errors will be negligible in the end result is technically possible. It gave a definite idea of the magnitude of the error produced when various environmental factors do vary, and this is very necessary in determining the probable limits of error.

There is a way of testing the conclusion that with the conditions controlled as suggested the constant error is negligible. If the same plants are measured during *similar portions* of successive periods of flowering activity, there is but one other obvious variable—total age of plant. If the latter has no measurable effect the two frequency distributions should duplicate. On this point I have no data, but Goodspeed and Clausen have corroborated the expectation in their conclusion number two. I do have some data on random samples of the same pure line grown in different years. This will be taken up later, however, as another point is involved.

Now the question arises: If records are made in this uniform manner, how many records from each plant are needed to obtain a measure of that plant with the precision necessary for a genetic investigation? Goodspeed and Clausen say that twenty-five flowers is the minimum. At the beginning of my Nicotiana investigations (1908), I used the same number, curiously enough. But I soon found that this was "accuracy with no significance," and the number was reduced to five. I now use but one measurement per plant. This is done because the precision is so nearly that of using twenty-five flowers, that it would be a waste of labor to try to attain the other. Furthermore the precision obtained by measuring twenty-five flowers is only appreciably greater when it can be done in a short time, otherwise *constant* errors may become very much greater.

The precision attained by measuring one flower per plant is all that is required for the use to which the data are to be put, and it is a rule of experimental physics not to strive for greater accuracy.

This matter can and has been tested in two ways. The first is to compare random frequency distributions of the corolla size of single plants with frequency distributions of the flowers when selected from vigorous branches and measured on the same day they have opened. This procedure gives a measure of the accuracy of single flower selections. To illustrate this, data from two species with very different sized flowers are submitted.

TABLE II

Comparison of Random Samples of Corolla Length on Single Plants and Samples in which Constant Errors have been Largely Eliminated

Name	Class Centers in Millimeters											
	20	21	22	23	24	25	26	27	28	29	30	31
N. paniculata, Random	...	...	...	...	1	3	16	2	3	...	...	....
" " Selected	...	...	...	...	...	4	18	3	...	...	...	....
" " Ran.	...	...	...	2	4	14	4	1	...	...	...	....
" " Sel.	...	...	...	...	5	17	3	...	...	...	...	....
" " Ran.	...	...	...	...	...	...	4	16	5	...	...	....
" " Sel.	...	...	...	...	...	...	3	20	2	...	...	....
" " Ran.	...	...	...	2	3	15	4	1	...	...	...	....
" " Sel.	...	...	...	...	3	22	...	...	...	...	...	....

Name	Class Centers in Millimeters											
		70	73	76	79	82	85	88	90	94	97	100
N. alata gr., Ran.	...	...	...	1	3	16	4	1	...	...	...	....
" " " Sel.	...	...	...	...	2	22	1	...	...	...	...	....
" " " Ran.	...	...	...	...	1	6	14	3	1	...	...	....
" " " Sel.	...	...	...	...	...	3	18	4	...	...	...	....
" " " Ran.	...	...	...	...	...	2	3	17	3	...	...	....
" " " Sel.	...	...	...	...	...	...	2	23	...	...	...	....

These plants are among the most uniform and the most variable respectively, and give an idea of the range of variability involved.

The other test made was to select fifteen flowers on a plant at random, and determine the mean to the nearest millimeter; then to find the deviation from this mean when single flowers were selected. In 100 tests of flowers shorter than thirty millimeters 88 selections were made within the 3 millimeter class to which the mean belonged. The remainder were in contiguous classes. On flowers between 70 and 100 millimeters long 82 out of 100 selections were within the 6 millimeter class to which the mean belonged. The remainder with 2 exceptions were in contiguous classes.

From these tests it will be seen that the probable error of the selection (equal chances) is not over plus or minus 2 percent. If this were a constant error it would be considerable. But it must be remembered that it belongs to the class of accidental errors and that in the long run the minus errors are compensated by the plus errors.

Such compensation can be clearly seen and the accuracy of the method perhaps most clearly demonstrated by comparing frequency distributions of the same pure line, daughters of the same plant, during successive seasons. In a number of cases populations of sister plants were grown for two and three years. The seed in each case came from single 1909 or 1910 plants, and since the percentage germination remained practically constant, the different populations are in the nature of duplicate and triplicate determinations. If then the frequency distributions are sufficiently alike that they may be presumed to be random samples of one population, the method is accurate enough for genetic purposes. A sample of the result is shown in Table III.

TABLE III

Random Samples of the Same Population Grown in Different Seasons

Name	Class Centers in Millimeters												Means
	34	37	40	43			85	88	91	94	97	100	
N. longiflora, var. *A*, 1911	...	13	80	32	...	...	...	...	...	...	...	...	40.46±.11
“ “ “ “ 1912	1	4	28	16	...	...	...	...	...	...	...	...	40.61±.19
“ “ “ “ 1913	...	4	32	1	...	...	...	...	...	...	...	...	39.76±.12
N. longiflora, var. *B*, 1911	...	...	...	...	...	...	...	6	22	49	11	...	93.22±.16
“ “ “ “ 1912	...	...	...	...	...	...	...	2	16	32	6	1	93.37±.20
“ “ “ “ 1913	...	...	...	...	...	...	...	5	7	10	2	...	92.12±.37

When one takes into consideration the difference in size of corolla among Nicotiana species and varieties that will cross and give fertile hybrids—*i. e. N. langsdorffii* 21 mm. and *N. alata grandiflora* 85 mm., it is scarcely necessary to enter into a biometrical argument on the precision of the method. Here are two small samples of the same population of *N. langsdorffii* grown in 1911 and 1914:

Designation	Class Centers in Millimeters				
	19	20	21	22	23
1911 plants....................	....	3	12	1	2
1914 plants....................	1	9	33	7	1

Can it be doubted that the phenotype for corolla length to which *N. langsdorffii* belongs is shown here with an accuracy much greater than is necessary when an analysis of the hybrid progeny of it and *N. alata grandiflora* is contemplated? Biometrical methods are much too imperfect to demand more. There is no intention to discuss here the reasons why the biometrical methods in general use in genetics are imperfect. But it must be emphasized that they are merely used in default of better, since many of them cannot be defended either mathematically or biologically. For example, common sense tells us that equal-sized classes should not be used for the two very different species shown in Table III, where the corolla of one is three times that of the other, yet no satisfactory method has been proposed which does away with the difficulties involved. Since it is necessary to use such poor methods in calculating our end results in genetic studies of size, however, one should remember that labor to record data far more precisely than these methods require is *labor wasted.*

At the same time, though one may believe that biometrical methods are imperfect for certain purposes, they are founded on the theory of probability and when used should be used with this in mind. Having recorded his data with the precision desired, one should not try to analyze them until he has collected a sufficient number of observations to make calculations of residual errors have meaning. Just what the minimum number should be varies with the problem and cannot be discussed in this paper. There are several textbooks on the Theory of Measurements in which the matter is treated in detail. All I wish to point out here is that in every problem capable of biometrical analysis there is such a minimum, and if the data to be analyzed are far under this required minimum, no over precision (in cases where this is possible) in making the records will give them value.

An excellent illustration of this is found in Goodspeed's third article on Quantitative Studies of Inheritance in Nicotiana Hybrids.[1] The author used his method of recording measurements of flowers through a considerable portion of the flowering season in order to determine the phenotypes to which the plants belong, and yet has made analyses of frequency distributions having such a small number of entries that they possess no meaning whatever. Among 44 frequency distributions, 29 have less than 12 plants recorded. He recognizes the fact that the number of plants involved is too small, but feels that this deficiency is balanced by the accuracy of his records.

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[1] Univ. Cal. Pub. Bot. 5: 223–231. 1915.

He says: "Data which have been submitted, however, leave no room for doubt in my own mind that investigations on the inheritance of flower-size demand the recognition of certain definite criteria and that the results of such investigations are vitally influenced by inherent as well as externally induced physiological states peculiar to the plant. Thus it remains to be seen if as many as 800 plants are necessary to establish the validity of an expanded Mendelian notation in F_2 of a flower-size hybrid, whether the 40,000 to 80,000 measurements, seemingly essential to a fair expression of results, can be accumulated. In other words, the experiment with which this paper deals has been a partially successful effort to measure many flowers on a few plants with the thought that the conception of flower-size would thus be approximately perfect for a few, rather than certainly imperfect for many plants. It is undeniably true that the number of plants is smaller than it should be, and it is perfectly evident that if the flowers on a larger number of plants cannot be correctly measured the attempt is not worth making."

One could hardly find a better illustration of "accuracy without significance." These views are absolutely indefensible mathematically. It has been shown that the method used by Goodspeed in making his records has only a fallacious claim to great precision; but, granting that the method is extremely accurate, it is an accuracy unnecessary to the end result. On the other hand, it should be clear that records in sufficient number to make probable errors significant is positively essential for a biometrical analysis. This end can only be attained by recording larger numbers of plants and not by over-refinement in the plant records. The plant records should have the precision required by the end result, but greater precision does not influence this result.

Harvard University

RELATION OF OXIDASES AND CATALASE TO RESPIRATION IN PLANTS

CHARLES O. APPLEMAN

The chemical mechanism of respiration in plants is very complex and imperfectly understood. It is a product of the living cells and is capable of bringing about, at low temperatures, the oxidation of organic substances which, in the laboratory, are oxidized only by employing very high temperatures and powerful reagents.

The presence of oxygen activators and carriers in plants has long been recognized and Traube as early as 1877 called these substances oxidizing ferments. Bertrand[6] was led to believe that the oxidizing ferments are more or less specific in their action, so he proposed the term oxidases as a group name for these ferments. Oppenheimer[11] has classed the oxidation enzymes according to the substances now generally recognized as acted upon by these enzymes. He realizes, however, the provisional character of such a classification and states further that the oxidation ferments represent the darkest Africa in the ferment world. The phenolases of Oppenheimer's classification accelerate only the oxidation of aromatic substances and are thus the oxidizing enzymes that are measured by the oxidase reagents in common use at the present time. The oxidases referred to in this paper include only the phenolases of Oppenheimer's classification.

Practically all attempts to explain the mechanism of respiration have assigned these long-established oxidative forces in the cell a rôle in this process, although such a relationship is almost entirely hypothetical. The chief difficulty lies in the fact that the oxidases, as detected and measured by the prevailing methods, have no direct action on the ordinary substances consumed in respiration, as sugar, for example. When it was recognized that respiration occurs in two distinct stages: namely, an anaerobic and aerobic stage, the oxidases were relieved of the responsibility of direct oxidation of these complex organic substances; since on this basis they would be concerned only in the oxidation of the decomposition products of the anaerobic stage. But all the known products of this stage are aliphatic substances incapable of being directly oxidized *in vitro* by the oxidases; therefore, the situation remains nearly as perplexing as ever.

The very ingenious hypothesis elaborated by Palladin[12] and his co-workers to explain the mechanism of respiration comes the nearest of anything yet offered to overcome the chief difficulties encountered in ascribing to the oxidases a function in respiration. It must be remembered, however, that this explanation is still in the hypothetical stage.

There are certain substances in probably all plant and animal cells which have the power to decompose hydrogen peroxide with the evolution of molecular oxygen. Loew[9] thought that this action on hydrogen peroxide is due to a special enzyme to which he gave the name catalase. When one considers the abundance and wide distribution of catalase in plant and animal tissues, it is natural to suppose that it plays some important rôle in metabolism. But so far its function has not been definitely established. Much theory, based upon rather scanty experimental data, has attempted directly or indirectly to connect catalase activity with the oxidative forces of the cell. The work of Lesser[10] seems to furnish the most conclusive evidence in this direction. He made a large number of catalase determinations in different small animals and in different organs and tissues of the same animal and concluded that catalase is connected with physiological oxidations. Although a strict interpretation of his results does not show a casual relation between catalase and cell oxidation, it does show a remarkable correlation. Zieger[13] also made a study of the catalase content of nearly all groups of animals except the protozoa. He did not succeed in establishing a relationship between intensity of respiration and catalase activity, but he did show that there is some relation between catalase content and metabolism. He brought out this fact by the study of the catalase content in organs which are very active chemically, as the liver and kidney. The evidence from the plant side, for such a relationship, is indirect and inconclusive. Kohl[8] indirectly connects catalase activity with respiration by his claim that it functions in alcoholic fermentation.

In conformity with the literature both the oxidases and catalase have been spoken of as enzymes although in the light of our present knowledge their place in the category of enzymes is extremely doubtful. It is questionable whether they are even definite chemical substances. It may be more correct to speak of oxidase and catalase activity rather than oxidases and catalase. This paper, however, is not concerned with the chemical nature or mode of action of these substances, nor is it concerned with any particular theory of respiration. Therefore, an exhaustive citation of the literature is not pertinent to the matter in hand. The sole object in view was a quantitative study of the relation of both oxidase and catalase activity to intensity of respiration. Potato tubers seemed especially favorable material for a study of this kind, since respiration in these tubers is greatly accelerated by various artificial treatments and is subject to fluctations under natural conditions, as greening, sprouting, etc. The rate of respiration also varies in different parts of the same tuber and tubers of different varieties. Besides, these tubers contain very active oxidases and catalase. The modification of the intensity of respiration in the tubers was determined and at the same time measurements were made of both the oxidase and catalase activity in the juice.

METHODS

Respiration: The rate of respiration was determined by the amount of CO_2 expired from the tubers. No attempt was made to control the temperature, but all measurements that are compared were made at the same time and under identical conditions. Tubers of about a kilo's weight were allowed to respire twenty-four hours for each determination.

Oxidase: A manometric method was used for the oxidase determinations, the oxygen absorbed during the reaction being measured by the change of pressure within Bunzel's simplified apparatus. Both pyrocatechin and hydrochinon were first employed as the oxidizable substance, but it was soon found that they showed the same general relations in respect to oxidase activity under different conditions. Since the reaction with hydrochinon was very slow as compared with that of pyrocatechin, its use was soon abandoned in favor of pyrocatechin as the sole reagent. From Bunzel's[4] work on the "Oxidases in Healthy and in Curly-Dwarf Potatoes" in which he used 18 ring compounds, it may be concluded that the use of one favorable compound of this nature would give just as valuable comparative results as use of a larger number. He found a wide variation in the amount of oxygen absorbed by the different compounds, but aside from one or two exceptions, they all showed the same general relation of oxidase activity in the different juices examined. In another paper, Bunzel[5] states: "No matter what the derivation of the plant juice is, the relative oxidizability of the various compounds is approximately the same."

Bunzel's[3] apparatus, of the size now on the market, required very small amounts of juice for a determination of the oxidase in the tubers of the principal variety employed in this work. In order to avoid errors in the measurement of extremely small quantities of juice, resort was made to the following dilution method: 2 cc. of juice were measured into 10 cc. of distilled water and after thorough mixing, without violent shaking, 2 cc. of the dilution were placed in the long arm and 5 milligrams of oxidizable substance in the short arm of the apparatus. The determinations were made in a constant temperature box of 33° C. During the reaction the apparatus was shaken constantly at the rate of 180 complete excursions per minute. After repeated trials with the diluted juice, it was found that the rate of shaking within wide limits had no effect on the total amount of oxygen absorbed; but the velocity of the reaction seemed to be greatest at about the amount of shaking decided upon for the standard in this work.*

The manometer readings recorded in this paper are those made at the end of one hour, unless otherwise noted, and represent the oxidase activity in .33 cc. of undiluted juice. Although in the case of pyrocatechin a very slow oxidation continued for several hours, it came to a comparatively definite end point after an hour.

Catalase: The potato juice for the catalase determinations was prepared by grating the tubers with calcium carbonate in the manner described by the writer[1] in a previous paper. The calcium carbonate neutralizes the free acids which very rapidly destroy the catalase in the juice. The catalase measurements were made in the same kind of apparatus used for the oxidase measurements, except that the apparatus was graduated to read positive pressures. The juice for a catalase determination was diluted in the following manner: 2 cc. of juice were added to 25 cc. of distilled water. The juice was thoroughly mixed with the water by rotating the flask 25 times. One cubic centimeter of the mixture was carefully measured into the long arm of the apparatus and 1 cc. of Oakland dioxogen (hydrogen peroxid) in the short arm. The determinations were made in the same constant temperature box and shaken with the same shaker used for oxidase. The manometer reading at the end of five minutes constant shaking was considered the measure of catalase activity in .074 cc. of juice. All the catalase results, in the experimental part, represent the activity in this amount of juice, except when otherwise noted. The oxidase and catalase measurements were all made in duplicate and those whose results were not in close agreement were discarded.

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* The shaking machine is described in bulletin No. 191 from the Maryland Agricultural Experiment Station.

It was very desirable to use the same lot of juice for both catalase and oxidase measurements, but before this was possible it was necessary to determine what effect the $CaCO_3$ would have upon the oxidase activity. To this end, tubers were cut in half longitudinally so that each piece contained exactly one half of the seed and stem ends. One piece was grated without $CaCO_3$ and the other by dipping into $CaCO_3$ in the usual way for catalase determinations. Oxidase measurements were then made in the juice thus prepared. The average of seven determinations was exactly the same in both cases. Therefore, it is quite evident that the $CaCO_3$ exercises no appreciable effect upon the oxidase activity in potato juice, according to the method here employed for its measurement.

The above conclusions regarding the effect on oxidase activity of $CaCO_3$ in the juice apply only with pyrocatechin. The effect may be otherwise with other chromogens. In fact, the presence of $CaCO_3$ in the juice seems to depress the oxidation of aloin, while the peroxidase activity is actually stimulated both in the case of aloin and guaiaconic acid.

Experimental Results

Ethyl Bromide: In a previous paper the writer[2] has shown that short exposures to ethyl bromide gas will about double the respiration in old McCormick potatoes. This treatment was repeated and catalase and oxidase determinations made on the same tubers used for respiration. Duplicate determinations on untreated tubers were made at the same time and under the same conditions. The results show that the treatment has no effect whatever on the oxidases. The catalase activity on the other hand is greatly increased in the treated tubers.

TABLE I

Respiration of McCormick Potatoes After an Exposure of the Tubers to Ethyl Bromide Gas for Thirty Minutes

Date of Measurement	Time Elapsed After Treatment with Ethyl Bromide Gas	Milligrams of CO_2 per Kilo per Hour		Ratio
		Untreated	Ethyl Bromide Gas—30 Min.	
February 12	1 hour	19.17	37.94	1 : 1.98
February 24	12 days	16.60	18.54	1 : 1.27

TABLE II

Oxidase Activity in Juice from McCormick Potatoes After an Exposure of the Tubers to Ethyl Bromide Gas for Thirty Minutes

Experiment	Time Elapsed After Treatment with Ethyl Bromide Gas, Hours	Quantity of Juice Used, Cc.	Manometer Readings Expressed in Centimeters of Mercury	
			Untreated	Ethyl Bromide Gas—30 Min.
1	1	1	− 4.4	− 4.5
2	1	1	− 4.5	− 4.5
3	2	.5	− 3.0	− 3.2
4	24	.5	− 3.2	− 3.4
5	24	.2	− 1.0	− 1.0
6	48	.33	− 2.5	− 2.5
Average........	....	....	− 3.1	− 3.18

TABLE III

Catalase Activity in Juice from McCormick Potatoes After an Exposure of the Tubers to Ethyl Bromide Gas

Experiment	Time Elapsed After Treatment with Ethyl Bromide Gas—Hours	Manometer Readings Expressed in Centimeters of Mercury	
		Untreated	Ethyl Bromide Gas—30 Min.
1	20	+ 2.6	+ 3.5
2	21	+ 2.2	+ 3.6
3	40	+ 2.0	+ 3.5
4	40	+ 2.4	+ 3.5
Average	....	+ 2.3	+ 3.53

Cold Storage: If tubers are stored for a few weeks at low temperatures and then brought to room temperature, they respire much more rapidly than tubers of the same lot which were not subjected to the cold storage. The effect on the catalase and oxidase is almost identical with that under the ethyl bromide treatment, if the cold storage temperature does not fall below 3° C. In a previous work, the writer found that at temperatures around 0° C., or below, the catalase activity is actually less than in normally stored tubers. This was accounted for in the destruction of the catalase by free acids. The presence of free acids is demonstrated by the acid exudate from tubers stored at this very low temperature.

TABLE IV

Effect of Cold Storage on Respiration of McCormick Potatoes

Date of Measurement	Milligrams of CO_2 per Kilo per Hour		Ratio
	Tubers Stored at Room Temperature	Tubers Stored at 3° for 20 Days	
February 18..............	12.50	35.15	1 : 2.81

TABLE V

Catalase and Oxidase Activity in the Juice from McCormick Tubers after a Period of Cold Storage

Experiment	Manometer Readings Expressed in Centimeters of Mercury, Using .1 cc. of Juice for Catalase and .5 cc. for Oxidase			
	Tubers Stored at Room Temperature		Tubers Stored at 3° C. for 20 Days	
	Catalase	Oxidase	Catalase	Oxidase
1	+ 2.4	− 3.0	+ 4.2	− 2.9
2	+ 3.5	− 2.9	+ 4.5	− 2.7
3	+ 3.6	− 2.9	+ 4.2	− 2.3
4	+ 2.4	.....	+ 4.4	.....
Average........	+ 2.97	− 2.93	+ 4.32	− 2.63

Effect of Greening: Greening of potato tubers in light is a very familiar phenomenon. One of the physiological changes concomitant with the greening is a rise in respiration. This offered a good opportunity to make a quantitative study of catalase and oxidase in relation to a change in respiration naturally induced. In connection with other work, the writer found in different stages of greening, a much greater rise in respiration than in the experiments here recorded. Even with the degree of acceleration shown in Table VI there is an increase in catalase in the green tubers. But again, the oxidase activity is not increased with a rise in respiration; in fact, it is a little less in the green tubers than in the ungreened ones.

TABLE VI

Effect of Greening on Respiration, Catalase and Oxidase

Experiment	Milligrams of CO_2 per Kilo per Hour		Manometer Readings Expressed in Centimeters of Mercury			
			Catalase		Oxidase	
	Normal	Green	Normal	Green	Normal	Green
1	17.14	19.50	+ 2.5	+ 2.8	− 2.35	− 2.1
2	14.32	16.24	+ 2.1	+ 2.5	− 2.2	− 2.1

Effect of Sprouting: Whole tubers of many varieties produce sprouts only from the buds on the seed end. Tubers of such varieties were cut in half and respiration measured separately in the seed and stem halves. It was found that respiration is always much higher in the seed halves when the sprouts are left on. This difference seems to be greater during incipient sprouting than after the sprouts have attained considerable size. How much of the increased respiration in the seed ends bearing the sprouts is due to the respiration of the sprouts themselves is difficult to determine. If the sprouts are removed just prior to the measurement of respiration, the results are quite different and depend upon the variety in question. In all cases the difference in respiration between the seed and stem ends is suddenly reduced. In McCormick tubers, it always remains a little higher in the seed halves.

The behavior of the catalase activity in the two ends of the McCormick tuber is practically identical with that of respiration. No difference in the oxidase activity of the two ends could be detected by the Bunzel method, using either pyrocatechin or pyrogallol as the oxidizable substance.

TABLE VII

Comparison of Respiration in Seed and Stem Ends; McCormick Tubers, Sprouting Only From Seed Ends

	CO_2 per Kilo per Hour Expressed in Ratio of Seed and Stem Ends	
Sprouts	Seed Ends	Stem Ends
Not started	100	93.5
Not started	100	93.2
On during measurement	100	58.2
Removed prior to measurement	100	80.2
Removed prior to measurement	100	89.3

The results of a large number of colorimetric determinations, using aloin as the oxidizable substance, show that this method does not agree with the Bunzel method in indicating the relative oxidase activity in the two ends of McCormick tubers. The colorimetric method consistently showed a greater oxidase activity in the stem half of the tuber, although this half always exhibited a lower rate of respiration. Therefore, neither method disclosed any relation between oxidase activity and the intensity of respiration.

TABLE VIII

Catalase and Oxidase Activity in Seed and Stem Ends; McCormick Tubers Sprouting Only From Seed Ends

Date of Measurement	Average Length of Sprouts	Manometer Readings Expressed in Centimeters of Mercury			
		Seed Ends		Stem Ends	
		Catalase	Oxidase	Catalase	Oxidase
March 20......	0	+ 2.4	− 1.7	+ 2.1	− 1.9
March 22......	0	+ 3.1	− 2.1	+ 2.7	− 2.2
March 25......	8 mm.	+ 4.0	− 2.2	+ 3.1	− 2.2
April 6.........	12 mm.	+ 3.6	− 2.25	+ 3.0	− 2.1
Average........	....	+ 3.36	− 2.06	+ 2.72	− 2.12

Comparison of Different Varieties: Tubers from different varieties, but under identical storage and sprouting conditions, were found to possess different rates of respiration. This fact being established, experiments were next planned to determine if there is a corresponding

TABLE IX

Respiration of Tubers from Two Different Varieties. All Conditions of Experiment Identical in Both Cases

Date of Measurement	Milligrams of CO_2 per Kilo per Hour McCormick	Carman No. 1
April 21....................................	12.83	17.36

TABLE X

Catalase and Oxidase Activity in Juice from Tubers of Two Different Varieties

Date of Measurement	Manometer Readings Expressed in Centimeters of Mercury			
	Catalase		Oxidase	
	McCormick	Carman No. 1	McCormick	Carman No. 1
April 14........	2.3	3.8	2.2	.60
April 23........	2.85	3.15	2.6	.64

difference in either the catalase or oxidase activity in the varieties showing a difference in rate of respiration. A varietal influence on both catalase and oxidase was soon discovered, although it is much greater in some cases than in others. The most striking difference was noted in the case of McCormick and Carman No. 1. Both varieties were under identical storage conditions for nearly two months prior to the date of experiment and both bore sprouts of practically the same vigor as determined by length and total weight. The sprouts were removed just before the measurement of the rate of respiration. The Carman No. 1 tubers respired more rapidly than the McCormick tubers. The catalase activity in the two varieties was strikingly correlated with respiration. On the other hand, the oxidase activity was four times greater in the McCormick tubers. Tables IX and X show typical experiments with these two varieties.

Summary and Conclusions

The introduction contains a statement of the fundamental difference between physiological oxidation as it occurs in respiration and ordinary combustion of organic substances in air. This is followed by a brief discussion of oxygen activators and carriers in plants and the difficulties encountered in assigning to the oxidases a rôle in respiration.

The important literature pertaining to catalase in its relation to respiration is reviewed.

The task was a quantitative study of the relation of both oxidase and catalase activity to intensity of respiration. Potato tubers seemed especially favorable material for a study of this kind since respiration in these tubers is greatly accelerated by various artificial treatments and is subject to fluctuation under natural conditions, as greening, sprouting, etc. The rate of respiration also varies in different parts of the same tuber and in tubers of different varieties. Besides, potato tubers contain very active oxidases and catalase. The modification of the intensity of respiration in these tubers was determined and at the same time measurements were made of both the oxidase and catalase activity in the juice. The data seem to justify the following conclusions:

1. The oxidase content in potato juice gives no indication of the intensity of respiration in the tubers. In other words, there is no

correlation between oxidase activity and the rate of respiration in these organs. The author does not disclaim any rôle of the demonstrable oxidases in respiration, but they certainly are not the controlling factor in regulating the rate of respiration in potato tubers.

2. Catalase activity in the potato juice shows a very striking correlation with respiratory activity in the tubers.

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INFLUENCE OF CERTAIN SALTS AND NUTRIENT SOLUTIONS ON THE SECRETION OF DIASTASE BY PENICILLIUM CAMEMBERTII[1]

William J. Robbins

Much has been published in recent years concerning the regulatory production of enzymes, but the investigations have been largely confined to the influence of the organic compounds on the production and secretion of enzymes. Little attention has been devoted to the effects of the mineral elements on enzyme formation. It would seem that such studies are of importance for several reasons. They might lead, for example, to a better understanding of the origin of enzymes. They should lead also to a clearer realization of the rôles played by the mineral elements in plant nutrition. In this regard it is significant that there exists a relation between potassium and carbohydrate formation in green plants, and also a relation between calcium and starch translocation. The rôles played by potassium and calcium in these processes are not understood, but it is possible, and it has been suggested by some investigators, that they condition the formation of certain of the carbohydrases.

It is also conceivable that studies of the effect of mineral salts on the secretion of enzymes would be important in the realm of plant pathology, particularly in the problem of disease resistance in plants. The interpretation of the action of distilled water on plants may also be aided by such studies.

As a result of these considerations a problem was evolved comprehending an investigation of the influence of certain single salts, and of certain nutrient solutions with various modifications, on the secretion of diastase by *Penicillium camembertii* Thom. The modifications of the nutrient solutions consisted in the replacement of certain essential radicals of salts by nonessential radicals.[2]

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[1] Contribution, Laboratory of Plant Physiology, Cornell University.

[2] It is a pleasure to acknowledge the indebtedness of the author to Dr. Lewis Knudson for suggesting this problem, and for constant aid and assistance in the preparation of the manuscript.

At the outset of the investigation it was found that a considerable amount of experimentation would be necessary in order to obtain adequate methods of research. It was necessary to devise a new method for measuring starch digestion. It was also essential to determine the significance of the number of spores sown in its bearing on the rate of digestion, as well as the effect of various kinds of distilled water on the rate of digestion. Rather extensive data were obtained in the preliminary experiments, of which only the salient facts are presented.

METHODS AND MATERIALS

Glassware.—The vessels used in these experiments were all of Jena glass. They were first cleaned with soap and water and chromic acid cleaning mixture, rinsed well with tap water and distilled water, and finally rinsed with the water used in the experiments.

Chemicals.—The chemicals were all of high grade, either Baker's analyzed or Merck's highest purity. The starch used was Merck's soluble starch, which is prepared from potato starch according to Lintner's method as described by Allen (1909). A solution of this starch, when the solvent is redistilled water, permits a small amount of growth of *Penicillium camembertii.* This would seem to show that the starch contains traces of mineral nutrients. According to Ford (1904 *A*), such a preparation contains phosphate, and perhaps organic phosphorus, which cannot be completely removed. According to Thomas (1914), also, the phosphorus present in purified samples of starch is in organic combination.

Water.—The laboratory distilled water, which is derived by distillation from an iron boiler and which is stored in a block tin tank, contains a dark brown precipitate when the last few liters in the tank are drawn. This precipitate consists of some form of iron. The water showed by test with Nessler's reagent no ammonia, and by the diphenylamine reaction no nitrates. Redistilled water was prepared by the double distillation of this water from Jena glass flasks containing acid and alkaline potassium permanganate. This method is described by Jones and Mackey (1897). Water treated with carbon black was prepared by adding 90 grams of moist carbon black, G Elf brand, to 4 liters of distilled water, allowing it to stand for three hours with occasional shaking, and then filtering.

On comparing the growth of *Penicillium camembertii* in a solution of starch made in these three types of water, distinct evidences of toxicity were noted from the laboratory distilled water. The mycelium in this distilled water was knotty in appearance and in small tufts. The mycelium in redistilled water or in distilled water treated with carbon black was fluffy in appearance and in a connected mat. The apparent toxicity of the distilled water, due perhaps to the presence of iron noted above, led to the use of either redistilled water or distilled water treated with carbon black throughout the experiments.

On comparing the digestion of starch by *Penicillium camembertii* in the three types of water, it was found that this was generally the most rapid in redistilled water, and in most cases slowest in distilled water. The presentation of this phase of the subject is reserved for a future paper.

Sufficient carbon black for the entire investigation was purified at one time by washing it during a period of a week with nine changes of distilled water and two of redistilled water. The carbon black when used contained approximately 72 percent of water. The amount used per 4 liters of water was therefore equivalent to about 25 grams of dry material.

Analytical Methods

In the experiments with nutrient cultures the rate of starch digestion was determined by finding the number of days required by the fungus to digest completely the starch in the culture medium. Complete digestion was determined by the Katz (1898) method. This consists in brief of the removal, daily, of a drop of the culture fluid under antiseptic conditions and the determination of the presence of starch by the use of iodine. When the drop removed shows no coloration with iodine, digestion is considered complete.

In the experiments with single salts it was deemed preferable to determine the amount of starch digested at a given interval from the time of inoculation. This procedure is more accurate and less tedious than the Katz method, and has the advantage of permitting the determination of the starch digestion when the fungous mycelia of all the cultures of a given series are of the same age.

Difficulty was experienced, however, in finding a method suitable for determining how much of the starch originally present in a culture solution had been digested by a fungus growing therein. It was considered impracticable to determine the amount of starch digested by finding the amount of the reducing sugar produced. A part at least of the sugar produced by the action of diastase is used in the metabolism of the fungus. Furthermore, it has not been demonstrated that *Penicillium camembertii* changes to glucose all of the maltose formed by the action of its diastase on starch. An alternative method might be the following: to determine the reducing value of a part of the solution; to hydrolyze the carbohydrates present in the same volume of the solution by boiling with hydrochloric acid, and to determine the reducing value of this portion. The difference between these two determinations should be the value of the nonreducing carbohydrates of the solution expressed in terms of glucose. This method was considered impracticable because of its tediousness and because of the uncertainty of the nature of the reducing sugars formed from starch by *Penicillium camembertii*.

An attempt was therefore made to evolve a different method of determining diastatic action. It is known that starch and a part of the dextrins are insoluble in a concentrated aqueous solution of alcohol. The sugars formed as a result of the action of diastase on starch, and perhaps part of the dextrins, are soluble in such a solution. This fact was consequently employed in the new method of determining diastatic action here described.

As finally used the method is as follows: By means of a pipette, 20 cc. of the medium are added, slowly and with constant shaking, to 70 cc. of 95 percent alcohol, which is acidified with 1 cc. of hydrochloric acid (sp. gr. 1.18–1.19).[3] This is allowed to stand over night, then filtered through a Gooch crucible, dried at 105° C., cooled in a desiccator over anhydrous $CaCl_2$, and weighed. This method gives us directly the amount of starch and dextrins which have not been digested to the point at which they are soluble in 73 percent alcohol.[4]

The applicability of this method to the determination of diastatic action might be questioned. It was considered necessary, therefore, to compare the measurement of diastatic action by this method with the measurement of diastatic action as found by the amount of reducing sugar produced. This comparison was made by determining the influence of the quantity of diastase on starch digestion, and the influence of time on the digestion by diastase.

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[3] Weaker solutions of hydrochloric acid than this may be used. See below.

[4] A mixture of 70 cc. of 95 percent alcohol + 21 cc. of nonalcoholic liquid is a solution of about 73 percent alcohol.

Influence of Quantity of Enzyme.—Fifty cubic centimeters of a 2 percent (approximate) soluble starch solution was placed in each of seven 125 cc. Erlenmeyer flasks. The quantities of 0.1 percent Taka diastase solution used and of the water added in order to keep the concentrations of starch the same throughout are indicated in Table I. After approximately 30 minutes digestion at room temperature, 20 cc. were removed from each Erlenmeyer flask and the undigested starch and dextrins were determined by the alcoholic precipitation method given above. The results follow in Table I:

TABLE I

50 Cc. of 2 Per cent Soluble Starch Plus	Undigested Starch (Mg. per 20 Cc.)	Starch Digested (Mg. per 20 Cc.)	Starch Digested per 1 Cc. of Diastase Solution (Mg. per 20 Cc.)
0 cc. diastase + 32 cc. H_2O	208.0	.....	.....
1 cc. “ + 31 cc. “	194.9	13.1	13.1
2 cc. “ + 30 cc. “	179.3	28.7	14.3
4 cc. “ + 28 cc. “	153.0	55.0	13.7
8 cc. “ + 24 cc. “	114.8	93.2	11.6
16 cc. “ + 16 cc. “	101.7	106.3	6.6
32 cc. “ + 0 cc. “	47.8	160.2	5.0

From these data it would appear that the proportionality between the amount of Taka diastase present and the amount of soluble starch digested, as measured by this method, holds to the point where approximately 25 percent of the original starch has been transformed into substances soluble in 73 percent acid alcohol. This is in fair agreement, considering the fact that the starch and enzyme preparations employed were not purified, with the results obtained by Kjeldahl (1879), Henri (1903), and Ford (1904 *B*), who, working with different starch and enzyme preparations and under different temperature conditions, used the reducing power of the solution as a measure of diastatic action.

The results of the determinations summarized in Table I are presented in the form of a curve in figure 1. On the abscissæ the amounts of Taka diastase solution used are given, and on the ordinates the amounts of digestion. The line parallel to the base represents the starch content per 20 cc. of the original solution.

Influence of Time.—The Taka diastase used contained, according to digestion determinations, insufficient maltase to make certain the complete transformation of the maltose to glucose. With malt diastase,[5] however, it was found that 0.1 g. of diastase produced no evident hydrolysis of 0.3 g. of maltose in 7 hours at 24° C. With malt diastase as the hydrolyzing agent, therefore, a direct comparison could be made between diastatic action as measured by the maltose produced and diastatic action as measured by the material precipitated in 73 percent acid alcohol.

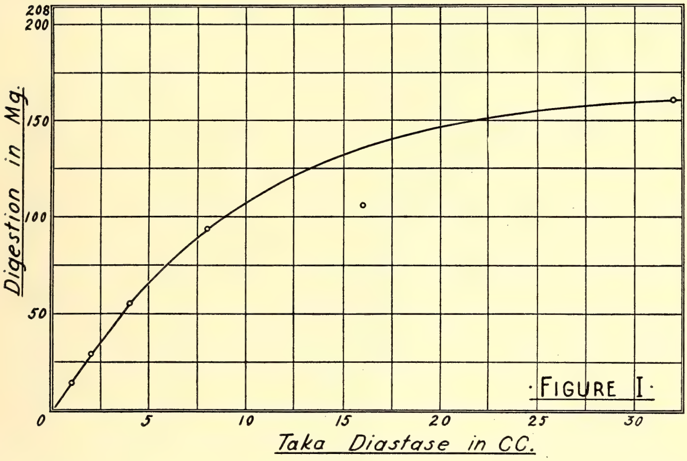

FIG. 1.

About 0.175 g. of malt diastase (Merck's medicinal) was dissolved in 20 cc. of water, and after being filtered to remove all undissolved particles this solution was added to 1,750 cc. of a 2 percent (approximate) starch solution. The mixture was kept in a constant temperature oven at 24° C., but was removed from the oven and replaced each time a determination was made. The data obtained are summarized in Table II:

It is evident that the digestion measured by alcoholic precipitation is greater than that measured by the maltose produced. In other words, a certain fraction of the digestion products is not maltose and

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[5] Merck's medicinal.

TABLE II

Time in Min.	Undigested Starch (Mg. per 20 Cc.)	Starch Digested (Mg. per 20 Cc.)	Starch Digested per Minute	Cuprous Oxide (Mg. per 20 Cc.)	Starch Digested Calculated from Maltose Determined (Mg. per 20 Cc.)	Starch Digested per Minute
0	344.1	.....	...	12.4	.....	...
16	334.5	9.6	.60	23.5	6.4	.4
29	325.4	18.7	.64	32.7	13.3	.46
44	314.9	29.2	.66	44.5	22.2	.50
60	305.5	38.6	.64	54.8	30.0	.50
80	304.7	39.4	.49	74.5[6]	44.7[6]	.51
100	276.7	67.4	.67	82.3	50.6	.51
123	264.9	79.2	.64	98.0	62.4	.51
151	242.5	101.6	.67	116.9	76.6	.51
185	229.6	114.5	.62	138.8	93.1	.50
245	192.5	151.6	.62	178.3	122.7	.50
323	164.2	179.9	.56	224.0	156.9	.48
420	122.1	222.0	.53	275.2	195.3	.46
549	85.6	258.5	.47	321.7	230.1	.42
702	69.6	274.5	.39	339.8	243.6	.35

is soluble in 73 percent alcohol, and the amount of this material increases with the time of digestion.

Calculating the digestion per unit of time, it would seem that, measured by either method, the amount of starch digested is proportional to the time for the first 245 minutes or until about 44 percent of the starch is in such form as is soluble in 73 percent alcohol. From that point on, the starch digested per unit of time steadily falls off.

The close correspondence of the two methods can be noted from the curves in figures 2 and 3, in which the time in minutes is given on the abscissæ and the amount of digestion in milligrams on the ordinates. In figure 2 the line parallel to the base at 344 mg. represents the starch content of the original solution per 20 cc. as determined by alcoholic precipitation. By acid hydrolysis and sugar determination the starch content of the original solution was found to be 367.6 mg. per 20 cc. In figure 2 the results are plotted for the entire 702 minutes, in figure 3 for the first 151 minutes only. The latter figure shows clearly that, under the conditions of the experiment, in the earlier stages of diastatic action the amount of starch digested, measured by either method, is proportional to the time.

It seemed probable that the difference between the digestion, as determined by the maltose produced and by the material precipitated in acid alcohol, could be largely overcome if a concentration of alcohol stronger than 73 percent were used as the precipitating agent. The influence of time on diastatic action was therefore determined by precipitating the starch and dextrins in 86 percent, in 73 percent, and in 60 percent acid alcohol. As was expected, the stronger the alcohol the greater was the precipitate. The close parallelism, however, between the curve for the precipitates in 86 percent alcohol and the curve for the precipitates in 73 percent alcohol seemed to justify the use of the weaker strength. It is advantageous to use 73 percent alcohol (70 cc. of 95 percent alcohol + 1 cc. of HCl + 20 cc. of the solution) because the smaller quantity of liquid facilitates filtration and other mechanical operations incident to the determination.

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[6] Time for this determination was 87.5 min.

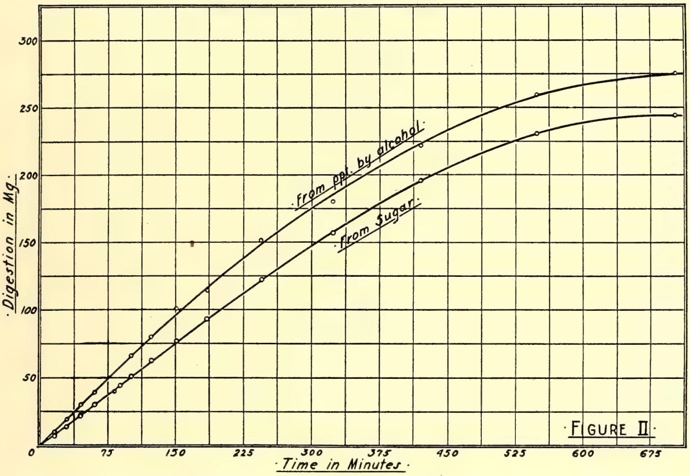

FIG. 2.

In all the determinations the alcohol was acidified with HCl in order to facilitate the flocculation of the starch and to prevent the precipitation of the salts present. It was found by experiment that the concentration of the HCl used is more or less a matter of indifference. The amount of precipitate at various stages of digestion in alcohol acidified with 1 cc. of HCl (sp. gr. 1.18–1.19), and in alcohol acidified with 1 cc. of acid one third of this strength, were identical.

From a consideration of the preceding data it would seem that the precipitation of starch and dextrins in 73 percent acid alcohol is a method that can be used with considerable satisfaction for the determination of diastatic action.

Preparation of Cultures and Use of the Alcoholic Precipitation Method for Determining Digestion.—The culture solutions in which single salts were used were prepared as follows: One liter of a solution of M/100 concentration of the salt to be tested was prepared, and a stock solution of 1.6 percent soluble starch was also prepared by heating in an autoclav at a pressure of 15 pounds. The various concentrations of the salt—M/1000, M/10,000, etc.—were made by diluting the original salt solution and mixing with the starch solution so that the starch content of each series was approximately constant. Each culture contained 50 cc. of solution in an Erlenmeyer flask of 125 cc. capacity. The cultures were sterilized in an autoclav at 15

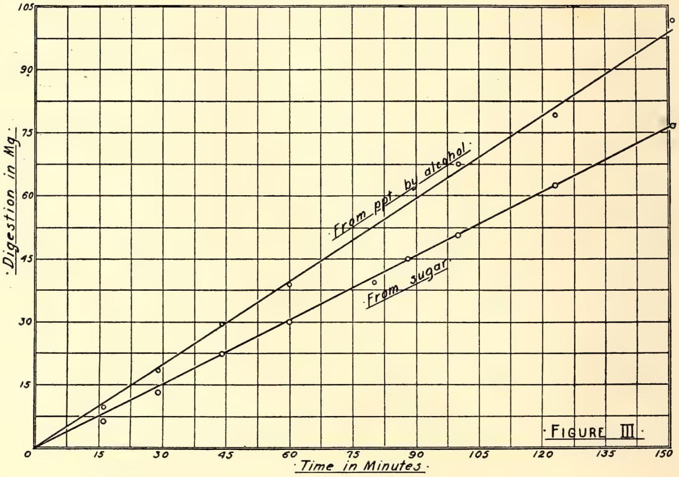

FIG. 3.

pounds pressure for 15 minutes. The culture solutions and vessels were weighed before sterilization, and just before analyzing the solution, each flask with its contents was brought up to the original weight by the addition of water. This was necessary in order to correct the change in concentration of the solution which results from the evaporation of water through the cotton plug of the flask.

The analysis of the original starch content was made after sterilization. After growth had occurred, the fungous mycelium was removed by filtering into a Gooch crucible to determine its dry weight after the method of Knudson (1913). The filtrate was caught in a 100 cc. test tube. Twenty cubic centimeters of this filtrate were removed and the undigested starch was determined by the alcoholic precipitation method described. The acidity or alkalinity of the filtrate to phenolphthalein and methyl orange was noted. All determinations were made in triplicate except the determinations of the original starch content, which in most cases was made in duplicate.

Method of Inoculating Cultures.—In inoculation the spores from the stock culture of the fungus were transferred by means of a sterile platinum needle to a second test tube containing sterile redistilled water. An equal number of drops of this spore suspension was added by means of a sterile pipette to each culture flask. This method of inoculation removes the danger of transferring soluble and suspended matter derived from the original stock culture to the experimental culture medium, as occurs in the method described by Hasselbring (1908). This modification was found necessary in view of the extreme sensitiveness of the fungus used to traces of inorganic nutrients, and of the possibility of introducing fragments of mycelium and organic matter. It was found that the same number of drops of the spore suspension must be used for each flask in a given series, because, within limits, the number of spores used in inoculation influences the amount of digestion. This is shown by the following experiment:

A set of 24 cultures was prepared, containing 0.8 percent of starch in distilled water which had previously been shaken up with cane sugar charcoal. One half of these cultures was inoculated with 3 drops of a suspension of spores of *Penicillium camembertii* in sterile distilled water, while to the other half 21 drops of the suspension were added. The results summarized in Table III are the average of triplicate cultures:

TABLE III

Soluble Starch in	Duration in Days	Original Starch Content (Mg. per 20 Cc.)	Starch Content After Digestion (Mg. per 20 Cc.)	Starch Digested (Mg. per 20 Cc.)	Dry Weight of Mycelium (Mg.)	Mg. of Starch Digested per Mg. of Dry Weight
3 drops of inoculum	7	141.9±.7	112.0±1.3	29.9	2.5±.1	11.9
21 drops of inoculum	7	141.9±.7	93.6± .9	48.3	2.3±.1	21.0
3 drops of inoculum	14	141.9±.7	79.7± .8	62.2	3.5±.1	17.7
21 drops of inoculum	14	141.9±.7	51.3± .8	90.6	3.1±.1	29.2

It is evident that any great difference in the number of spores used in inoculating the medium will produce considerable difference in the digestion, even though it may produce no effect on the detectable amount of mycelium formed.

INFLUENCE OF INORGANIC ELEMENTS ON THE SECRETION OF ENZYMES

Historical

Though considerable work has been reported by Katz, Went, Euler, Dox, Knudson, Kylin, and others on the relation of organic substances to the production and secretion of enzymes, relatively little has been reported on the relation of inorganic substances to the production and secretion of enzymes.

Fernbach (1890) concluded that the invertase formation by yeasts was influenced more by the source of nitrogen than by the source of carbon.

Effront (1902) states that phosphates, which influence yeast very favorably, are, on the contrary, unfavorable to the formation of invertase.

Saito (1910) investigated the formation of diastase by *Aspergillus Oryzæ* when grown in nutrient cultures containing either glucose, fructose, sucrose, maltose, galactose, lactose, or glycerol as sources of carbon, and either Witte's peptone, tyrosine, leucine, alanine, glycerol, asparagin, urea, ammonium tartrate, ammonium oxalate, ammonium chloride, ammonium sulfate, ammonium nitrate, dihydrogen ammonium phosphate, potassium nitrate, or calcium nitrate as sources of nitrogen. He tested the nutrient medium for diastase, and if it was lacking there he examined the mycelium for its presence. With nitrogen supplied to the nutrient solution in organic combination, diastase was always produced. With ammonium sulfate or ammonium chloride as the source of nitrogen, diastase was not formed, save when starch was the source of carbon, in which case the enzyme was found only in the mycelium. Saito concluded that the source of nitrogen is significant in the formation of diastase.

Stoward (1911) grew barley embryos for from four to eight days in gelatine, and found that a mixture of asparagin and mineral salts, consisting of $CaSO_4$, KCl, $MgSO_4$, KH_2PO_4, and $FeCl_3$, influences the secretion of diastase more favorably than either the asparagin or the mineral salts alone. After determining the effect of the mineral salts on the activity of the secreted diastase, he concludes that they enter in some way into the metabolism of the embryo and thereby influence its secretory function.

Javillier (1912) noted that *Aspergillus niger*, grown in Raulin's solution lacking zinc, secreted invertase sufficiently rapidly to invert saccharose, but that the quantity secreted, calculated per unit of dry weight of the fungus, was noticeably less than was produced by the fungus in a complete solution.

Euler and Meyer (1912) suspended yeast cells in solutions of various substances for times varying from 20 to 150 hours, filtered off the yeast, and determined the inverting power of a unit weight of this living yeast. They found that by suspending the yeast in a solution of 4 g. of asparagin, glycocoll, or ammonium sulphate in 500 cc. of Lintner's solution, the effect on the formation of the enzyme invertase was beneficial and the same in each case.

Euler and Cramer (1913), working with the same problem, state that invertase formation is clearly bound up with the new formation of protoplasm. The building up of protoplasm, however, is dependent on, first, the fermentation that supplies energy, and second, the presence of suitable nitrogenous material in the medium. When the solution used in the treatment of the yeast contains no nitrogenous material, invertase formation occurs, but in slight amount.

It is evident that information on the problem of the influence of inorganic salts on enzyme secretion is extremely meager.

THE EFFECT OF SINGLE SALTS ON THE DIGESTION OF STARCH BY PENICILLIUM CAMEMBERTII

The effect of the chlorides, the sulphates, the dihydrogen phosphates, and the nitrates of sodium and potassium, and of the chlorides, the sulphates, and the nitrates of calcium and magnesium, when present singly in solution, on the growth of *Penicillium camembertii* and on the digestion of starch by that fungus, was determined after the methods given above.

KCl and NaCl.—In this experiment various concentrations of KCl or NaCl were used. The fungus was grown in the dark at 25° C., and the amount of starch digested was determined at the end of one and of two weeks. The results summarized in Table IV are the averages for triplicate cultures. Where no probable error is given for these values, it is less than 0.1 mg.

Table IV

KCl and NaCl

Soluble Starch in	Duration in Days	Original Starch Content (Mg. per 20 Cc.)	Starch Content after Digestion (Mg. per 20 Cc.)	Starch Digested (Mg. per 20 Cc.)	Dry Weight of Mycelium	Mg. of Starch Digested per Mg. of Dry Weight
M/1000 KCl......	7	134.1± .8	103.8± .4	30.3± .9	1.5±.1	20.2±1.4
3M/10,000 KCl ...	7	133.1± .9	99.5±1.3	33.6±1.6	1.4±.1	24.0±2.0
M/100,000 KCl ...	7	133.5± .1	101.2±1.4	32.3±1.4	1.5±.1	21.5±1.6
Water treated with carbon black....	7	145.6±1.3	101.7±1.6	43.9±2.0	1.5±.1	29.2±2.3
M/100,000 NaCl..	7	143.8±1.0	103.4± .6	40.4±1.1	1.3	31.0±2.5
M/10,000 NaCl...	7	140.8± .1	104.1±3.0	36.7±3.0	1.5±.1	24.4±3.8
M/1000 NaCl.....	7	142.9	103.7± .9	39.2± .9	1.5±.1	26.1±1.8
M/1000 KCl......	14	134.1± .8	67.3± .7	66.8±1.1	2.0	33.4±1.8
3M/10,000 KCl...	14	133.1± .9	73.3± .6	59.8±1.1	1.8	33.2±2.0
M/100,000 KCl...	14	133.5± .1	71.1± .5	62.4± .5	1.7±.1	37.0±2.2
Water treated with carbon black....	14	145.6±1.3	58.5±1.	87.1±1.6	2.2±.1	39.6±1.9
M/100,000 NaCl..	14	143.8±1.0	67.4±1.5	76.4±1.8	2.2±.1	34.7±1.8
M/10,000 NaCl...	14	140.8± .1	69.2±1.8	71.6±1.8	2.1±.1	34.1±1.5
M/1000 NaCl.....	14	142.9	73.5±2.3	69.4±2.3	2.2±.1	35.1±1.8

In Table IV the probable errors have been calculated for the "starch digested," which is the difference of the two quantities "original starch content" and "starch content after digestion." They have also been calculated for the "mg. of starch digested per mg. of dry weight," which is the quotient of the starch digested in a culture divided by the dry weight of the mycelium produced in the same culture. In all later tables this calculation has not been made, though the probable errors are given for the averages of the determinations made on the original starch content, the starch content after digestion, and the dry weight of the mycelium.

From the data given in Table IV, it can be observed that, in general, KCl and NaCl decrease the amount of starch digested by *Penicillium camembertii*. This inhibition is evident in the case of both KCl and

NaCl at a concentration of M/100,000. The amount of salt producing an evident effect is very small. In the case of KCl, 50 cc. (the volume of each culture solution) of a M/100,000 solution contains about .000037 g. of KCl and the same concentration of NaCl contains a little less than .00003 g. That the effect of such a small amount of a neutral salt on the digestion of starch by a fungus could be measured might be doubted, if the results obtained at the end of the first week were not substantiated by the results obtained at the end of the second week.

It can also be observed that with increasing concentration of the salts the amount of starch digested is decreased, with three exceptions —M/100,000 KCl and M/1000 NaCl at the end of the first week, and M/1000 KCl at the end of the second week. None of these aberrant results, however, are verified in both determinations. This inhibiting effect of the salts on digestion is also evident when the loss per unit weight of fungus is considered, with the exceptions already referred to.

It may also be noted that in every case the absolute amount of starch digested is less in the presence of KCl than in the corresponding concentration of NaCl. The same is true with respect to the amount of starch digested per unit of dry weight of mycelium. Two exceptions to this last statement may be noted, namely, the M/1000 and M/100,000 concentrations at the end of the second week.

TABLE V

K_2SO_4 and Na_2SO_4

Soluble Starch in	Duration in Days	Original Starch Content (Mg. per 20 Cc.)	Starch Content After Digestion (Mg. per 20 Cc.)	Starch Digested (Mg. per 20 Cc.)	Dry Weight Mycelium (Mg.)	Mg. of Starch Digested per Mg of Dry Weight
M/1000 K_2SO_4	7	137.2 ± .9	113.7 ± 1.1	23.5	3.2 ± .1	7.3
M/10,000 K_2SO_4	7	136.3 ± .5	115.3	21.0	3.1 ± .1	6.8
M/100,000 K_2SO_4	7	139.8 ± 1.1	117.6 ± 3.2	22.2	3.0 ± .1	7.4
Water treated with carbon black	7	137.8 ± 1.3	111.5 ± .5	26.3	3.0 ± .1	8.8
M/100,000 Na_2SO_4	7	139.7 ± 1.2	114.9 ± .5	24.8	3.3 ± .1	7.5
M/10,000 Na_2SO_4	7	137.1 ± .3	109. ± 1.1	28.1	3.3 ± .1	8.5
M/1000 Na_2SO_4	7	135.9 ± .3	108.3 ± 1.5	27.6	3.4 ± .1	8.1
M/1000 K_2SO_4	15	137.2 ± .9	78.5 ± 1.2	58.7	4.0	14.7
M/10,000 K_2SO_4	15	136.3 ± .4	88.2	48.1	4.1	11.7
M/100,000 K_2SO_4	15	139.8 ± 1.1	75.6 ± .6	64.2	4.2 ± .2	15.3
Water treated with carbon black	15	137.8 ± 1.3	64.5 ± .8	73.3	4.0	18.3
M/100,000 Na_2SO_4	15	139.7 ± 1.2	76.0 ± 1.3	63.7	4.3 ± .1	14.8
M/10,000 Na_2SO_4	15	137.1 ± .3	76.4 ± 2.4	60.7	3.9 ± .1	15.6
M/1000 Na_2SO_4	15	135.9 ± .3	70.1 ± .9	65.8	4.0	16.4

K_2SO_4 and Na_2SO_4.—In this series *Penicillium camembertii* was grown in various concentrations of K_2SO_4 or Na_2SO_4. Incubation of cultures was made in the dark at 25° C. The data summarized in Table V are the averages of triplicate cultures.

From the data in Table V it may be noted that with K_2SO_4 and Na_2SO_4 much the same effect results as with the chlorides of the same bases. An inhibition in the actual amount of starch digested is produced even by a concentration of M/100,000. The effect of increasing concentration of the salts on the digestion is not so evident as with the chlorides. The M/1000 concentrations are clear exceptions, the digestion being increased over M/10,000 in both cases and in both weeks. This would appear to be a real effect, not one due to errors in determination. It can also be observed in this case that the K_2SO_4 has a greater inhibitory effect on digestion than the Na_2SO_4. This is shown in every case but one, the M/100,000 concentrations at the end of two weeks, in which the difference is very slight.

KNO_3 and $NaNO_3$.—*Penicillium camembertii* was grown in this case in the concentrations of KNO_3 or $NaNO_3$ noted in Table VI. The cultural conditions were those already noted. The results given in Table VI are the averages of triplicate cultures:

TABLE VI

KNO_3 and $NaNO_3$

Soluble Starch in	Duration in Days	Original Starch Content (Mg. per 20 Cc.)	Starch Content After Digestion (Mg. per 20 Cc.)	Starch Digested (Mg. per 20 Cc.)	Dry Weight of Mycelium (Mg.)	Mg. of Starch Digested per Mg. of Dry Weight
M/1000 KNO_3......	7	140.9± .9	12.9±3.1	128.0	15.7±1.7	8.1
M/10,000 KNO_3....	7	137.4± .1	53.8±3.6	83.6	9.4± .3	8.9
M/100,000 KNO_3...	7	138.7± .1	74.1±2.2	64.6	3.7± .1	17.5
Water treated with carbon black.....	7	143.1±1.1	68.4± .5	74.7	3.7± .1	20.2
M/100,000 $NaNO_3$..	7	136.4± .1	66.1±1.4	70.3	4.0± .1	17.6
M/10,000 $NaNO_3$...	7	137.8± .8	47.7±1.3	90.1	7.6± .3	11.8
M/1000 $NaNO_3$.....	7	131.8± .1	13.1± .8	118.7	7.2± .1	16.5
M/1000 KNO_3......	14	140.9± .9	Complete digestion		32.1± .4	——
M/10,000 KNO_3....	14	137.4± .1	10.7±1.5	126.7	12.8± .1	9.9
M/100,000 KNO_3...	14	138.7± .1	36.1± .8	102.6	5.3	19.3
Water treated with carbon black.....	14	143.1±1.1	34.3± .6	108.8	4.7	23.1
M/100,000 $NaNO_3$..	14	136.4± .1	24.9±2.0	111.5	5.3± .1	21.0
M/10,000 $NaNO_3$...	14	137.8± .8	9.0± .8	128.8	10.0± .2	12.9
M/1000 $NaNO_3$.....	14	131.8± .1	Complete digestion		13.5± .2	——

With the nitrates of potassium and sodium (Table VI) there is an increased growth which is greater in the KNO_3 than in the $NaNO_3$. We see, however, that with one exception—that of M/100,000 $NaNO_3$ at the end of two weeks—the M/100,000 concentrations decrease the actual amount of digestion, even though the amount of growth is increased in those concentrations. If the digestion per unit weight of fungus is considered, it is evident that the digestion decreases with increasing amounts of salt.

It can also be observed here that with the exception of the M/1000 concentration, which might be accounted for by the great difference in growth in favor of the KNO_3 culture, there is less digestion than in the corresponding strength of $NaNO_3$. This is also evident in the digestion per unit weight of mycelium, in which the digestion per unit of dry weight in the M/1000 concentration of the two salts agrees with the general proposition.

KH_2PO_4 and NaH_2PO_4.—In this series the fungus was grown in the presence of various concentrations of KH_2PO_4 or NaH_2PO_4. The cultural conditions were as before noted. The results summarized in Table VII are the averages of triplicate cultures:

TABLE VII

KH_2PO_4 and NaH_2PO_4

Soluble Starch in	Duration in Days	Original Starch Content (Mg. per 20 Cc.)	Starch Content after Digestion (Mg. per 20 Cc.)	Starch Digested (Mg. per 20 Cc.)	Dry Weight of Mycelium (Mg.)	Mg. of Starch Digested per Mg. of Dry Weight
M/1000 KH_2PO_4........	7	137.1± .1	102.8±2.7	24.3	2.3±.1	10.5
M/10,000 KH_2PO_4......	7	140.3± .2	197.9±2.2	32.4	2.7±.1	12.0
M/100,000 KH_2PO_4.....	7	139.7±1.6	104.7±1.1	35.0	2.2±.1	15.9
Water treated with carbon black..........	7	134.3± .6	100.8±1.	33.5	2.0±.1	16.7
M/100,000 NaH_2PO_4....	7	140.9± .2	110.1±2.2	30.8	2.2±.1	14.0
M/10,000 NaH_2PO_4.....	7	139.8	101.8± .8	38.0	2.3±.1	16.5
M/1000 NaH_2PO_4.......	7	140.4	106.3± .5	34.1	2.4±.2	14.2
M/1000 KH_2PO_4........	14	137.1± .1	80.7±2.1	56.4	3.3	17.1
M/10,000 KH_2PO_4......	14	140.3± .2	69.6± .5	70.7	3.1±.1	22.8
M/100,000 KH_2PO_4.....	14	139.7±1.6	74.1± .8	65.6	3.3±.1	19.9
Water treated with carbon black..........	14	134.3± .6	67.6± .8	66.7	3.3±.3	20.2
M/100,000 NaH_2PO_4....	14	140.9± .2	73.9± .5	67.0	3.4±.1	19.7
M/10,000 NaH_2PO_4.....	14	139.8	70.6± .3	69.2	3.3±.1	21.0
M/1000 NaH_2PO_4.......	14	140.4	70.5±1.3	69.9	3.3	21.2

Analyzing the data given in Table VII, we note that M/1000 KH_2PO_4 is the only solution which produces marked decrease in the amount of starch digested as compared to the water culture. In fact, from the loss noted in the NaH_2PO_4 series, NaH_2PO_4 appears to increase the amount of starch digested.

Again the potassium salt permits less digestion than the sodium salt, with two exceptions—M/100,000 concentrations at the end of the second week. It would appear that this failure to inhibit the digestion as compared to the check is due to the effect of the acid, not the basic, radical of these salts.

Ca and Mg salts.—Experiments similar to the above were performed, in which the chlorides, the sulphates, and the nitrates of calcium and magnesium were compared. Space precludes the citation of the actual data obtained. It will be sufficient to say that the results were very similar to those obtained with the potassium and sodium salts of the same acid radicals. The addition of M/100,000 of either $CaCl_2$, $MgCl_2$, $CaSO_4$, or $MgSO_4$ to distilled water treated with carbon black is sufficient to decrease the actual amount of digestion. The effect of this concentration of these salts on the growth of the fungus is inappreciable. $CaCl_2$, $MgCl_2$, $CaSO_4$, or $MgSO_4$ of a concentration M/10,000 depresses the actual digestion more than does a M/100,000 concentration of the same salts. The effect of this concentration on the dry weight of the fungus is also inappreciable. The amount of starch digested in M/1,000 concentration of these salts is greater than the digestion in M/10,000 concentration, but it is not so great as in the distilled water treated with carbon black. M/1,000 concentration of these four salts also increases the amount of growth. The dry weight of the fungous mycelium is from 0.5 to 1 mg. greater in the M/1,000 concentration than in the distilled water treated with carbon black or in the M/100,000 or M/10,000 concentration of these salts. The starch digestion per unit of dry weight is greatest in the distilled water treated with carbon black, and decreases with increasing concentration of the salt added. The addition of $Ca(NO_3)_2$ or $Mg(NO_3)_2$ to a solution of soluble starch in distilled water shaken up with carbon black increases the amount of digestion. It also increases the dry weight of the fungus. The digestion per unit of dry weight of mycelium, however, is less in the presence of the salt than in the water alone, and decreases with increasing concentration of the salt.

Discussion.—The results of this investigation give no support to the idea that potassium and calcium are intimately connected with diastase formation. Neither would it appear that sulphur, chlorine, magnesium, and sodium are closely connected with diastase formation. The addition of traces of the salts containing these elements produces no increase in the rate of digestion of starch. It is recognized, of course, that an abnormal condition is presented for the growth of the fungus when only a single salt is supplied, because the absence of other salts must be a limiting factor in the use of the one supplied. Nevertheless, if one of the nutrients mentioned above were specifically concerned in diastase formation, it might be expected that increased digestion would occur in its presence. No such increase is noted.

On the contrary, it has been found that the sulphates and the chlorides of potassium, sodium, calcium and magnesium in M/10,000 and M/100,000 concentrations decrease the rate of digestion. The cause of this inhibition is obscure.

There seem to be two possibilities: either the decreased digestion is due to an inhibition of the activity of the secreted diastase by the salt, or the effect is physiological; one of decreased secretion.

From a consideration of recent work on the effect of salts on the activity of diastase, the writer is led to believe that the effect is physiological. Recent work seems to show that if the salts at the concentrations used here have any effect on the activity of diastase, it should be one of acceleration of action rather than retardation. Hawkins (1913), working with malt diastase, determined the effect of NaCl and KCl, in concentrations varying from 2M to M/2,048; of $CaCl_2$, in concentrations varying from 1M to M/4,096; and of $MgCl_2$, in concentrations varying from M/2 to M/8,192. NaCl and KCl produced a retardation—15 percent at M/128, and 5 percent and 7 percent, respectively, at M/512—yet they had no effect at a concentration of M/2,048, and in all higher concentrations they produced a marked acceleration. $CaCl_2$, in all the concentrations used, accelerated the action of diastase; and $MgCl_2$, in all concentrations save M/8,192, which had no effect, also accelerated the digestion.

VanLaer (1913) states that in a medium of amphoteric reaction (à reaction amphotére), alkaline to methyl orange and acid to phenolphthalein, small quantities of the neutral electrolytes are indispensable to the manifestation of the properties of diastase.

In every determination of the reaction of the medium in this investigation where single salts were used, it was found that it was alkaline to methyl orange and acid to phenolphthalein. The determinations were made each time a culture solution was analyzed.

Sherman and Thomas (1915), working with a purified starch and a carefully prepared diastase, find an acceleration with NaCl, KCl, $NaNO_3$, Na_2SO_4, NaH_2PO_4, and KH_2PO_4 and state: "In our experiments it has been observed that as long as commercial starch, even of high grade, was used as a substrate, the smaller additions of the salts above mentioned have very little effect . . . ." It is therefore justifiable to assert that the chlorides and the sulphates of potassium, sodium, calcium and magnesium in M/100,000 and M/10,000 concentrations in distilled water treated with carbon black decrease the secretion of diastase. What the significance is of the fact that the potassium salts decrease the secretion more than do the sodium salts of the same acid radical, cannot be stated.

The fact that the addition of these salts to distilled water decreases the secretion of the enzyme diastase would seem to bear directly on the problem of the effect of distilled water on the growth of plants.

It is of interest to note in this connection that Merrill (1915) has recently found that boiling the distilled water in which the roots of seedlings have been immersed decreases its toxicity. The effect of the boiling has been ascribed by Merrill to a destruction of bacteria. It would seem possible that it might be due to the destruction of harmful enzymes or thermolabile toxines secreted in larger quantity in distilled water.

The conclusions reached by True (1914) on the leaching effect of distilled water on the roots of seedlings of *Lupinus albus* and the protective action of $CaCl_2$ on the growth of the roots also seem of particular interest. True concludes that in the presence of $CaCl_2$ the dissociating power of the distilled water over the proteids and other chemical mechanisms of the cell is largely undeveloped, and the chemical integrity of the cell is protected in some way unknown. Similarly it might be postulated that the salts used here prevent the separation of the enzyme from a union with the protoplasm. It should be stated, however, that True and Bartlett (1915) report that solutions of KH_2PO_4 and KCl act essentially like distilled water.

It has been noted that the phosphates of potassium and sodium do not inhibit the digestion, and that greatly increased digestion is obtained with the addition of the nitrates to solutions of starch in distilled water treated with carbon black. This might lend some credence to the view that phosphorus and nitrogen are connected with diastase formation. The results secured with phosphorus are, however, hardly definite enough to allow one to draw conclusions very rigidly. The increased growth in the presence of nitrogen may also explain the increased secretion as the secretion per unit of dry weight of mycelium produced decreases with increasing concentration of the nitrates.

DIGESTION IN NUTRIENT SOLUTIONS

The experiments on the influence of single salts revealed no evidence respecting the rôle of the elements in enzyme formation or secretion. It has already been stated that the absence of other salts might be limiting factors in the functioning of any given salt. Consequently, a series of experiments were performed to test the effect of the absence of various essential elements from the full nutrient solution.

In the first experiment a modification of Richards's (1897) medium[7] was employed.

The salts substituted were used in the same concentration as the originals and the substitutions made were as follows:

Minus	nitrogen,	KNO_3,	replaced	by	KCl
"	potassium,	KNO_3,	"	"	$Ca(NO_3)_2$
"		KH_2PO_4,	"	"	$Ca(H_2PO_4)_2$
"	phosphorus,	KH_2PO_4,	"	"	K_2SO_4
"	magnesium,	$MgSO_4$,	"	"	Na_2SO_4
"	sulphur,	$MgSO_4$,	"	"	$MgCl_2$
"	iron,	$FeCl_3$,	"	"	NaCl

Two series of experiments were performed, in one of which 50 cc. of solution and 0.4 percent of starch were used, and in the other 500 cc. of solution and 0.8 percent of starch. Redistilled water was used as the solvent. The cultures were grown in triplicate in the dark at room temperature, and the time required for complete digestion of the starch was determined by daily tests of the culture medium by the method of Katz (1898).

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[7] The medium used was composed of

KH_2PO_4	.5 g.
$MgSO_4$	.25 g.
KNO_3	1 g.
$FeCl_3$	Trace
Water	100 cc.
Starch	as indicated.

Though the dry weight of the mycelium was not determined (as each culture showed complete digestion), the amount of mycelium formed in all cultures, with the exception of that in the medium lacking iron, was noticeably less than in the full nutrient solution. The mycelium was least in the culture lacking all nutrients and in the culture lacking nitrogen. The growth in the culture lacking potassium differed in appearance from that in the other cultures in consisting of a large number of small bunches of mycelium, and not a continuous fluffy mass.

Certain differences were noted between the two sets of cultures in the relation of fruiting to digestion. All the cultures of the set containing 50 cc. of solution completed digestion before fruiting. In the set in which 500 cc. of solution was used, the full nutrient culture, and the cultures lacking phosphorus, sulphur, and iron, fruited before completing digestion, while the cultures lacking magnesium, nitrogen, and potassium did not. It will be noted from the results of these experiments, which are summarized in Table VIII, that at room temperature *Penicillium camembertii* digests 0.2 g. of starch in 50 cc. of modified Richards's solution in 9 days. A deficiency of iron, sulphur, magnesium, and phosphorus has little effect on the time required for digestion. A lack of nitrogen, on the one hand, and of all nutrients, on the other hand, has approximately the same effect, tripling the time as compared to that of the full nutrient culture in which .2 g. of starch in 50 cc. of solution is used, and increasing the time from sixteen to nineteen times when 4 g. of starch is contained in 500 cc. of solution.

TABLE VIII

Medium	Time for Digestion in Days 50 Cc. of Solution .4 Percent of Starch	 500 Cc. of Solution .8 Percent of Starch
Starch only	17	171+
Full nutrient	6	9
Full nutrient minus nitrogen	15	143
Full nutrient minus potassium	117+	171+
Full nutrient minus phosphorus	8	10
Full nutrient minus magnesium	9	17
Full nutrient minus sulphur	8	18
Full nutrient minus iron	6	12

The digestion in the absence of potassium is exceedingly slow. It was believed at first that here was a relation between potassium and diastase secretion. This, however, is not the case, as is shown by the following experiment, in which a different solution[8] was employed and the rate of digestion of starch was noted both in the presence and in the absence of potassium. The time required for the digestion of the starch in 50 cc. of the medium containing no K nor Na, and of the starch in 50 cc. of the same medium containing KH_2PO_4 or NaH_2PO_4 as indicated in Table IX, was determined by the method of Katz. Triplicate cultures were grown in the dark at room temperature, and the dry weight of the mycelium was determined as each culture showed complete disappearance of the starch.

TABLE IX

50 Cc. of Medium Plus	Time for Digestion (Days)	Dry Weight of Mycelium (Mg.)
M/10 KH_2PO_4	6	14.7
M/100 KH_2PO_4	6	11.1
M/1000 KH_2PO_4	4	6.4
M/10,000 KH_2PO_4	6	10.6
M/100,000 KH_2PO_4	7	6.6
No phosphate	8	5.1
M/10 NaH_2PO_4	9	9.6
M/100 NaH_2PO_4	8	5.4
M/10,000 NaH_2PO_4	6	6.7
M/10,000 NaH_2PO_4	7	6.4
.4 percent starch only	11	Weight not taken

It can be noted from the number of days required for complete digestion given in Table IX that a deficiency of potassium has little effect on the time required for digestion. In the medium used here it requires *Penicillium camembertii* but 8 days to digest 0.2 g. of starch in a deficiency of both potassium and phosphorus, which differs by but one or two days from the time necessary for complete digestion when both these elements are present in abundance. It would therefore appear that the long period of digestion in the minus potassium cultures of Table IX is due not to the lack of potassium, but to some other cause. This cause is apparently an inhibitive action of the

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[8] The nutritive solution used was composed of

$Ca(NO_3)_2$	0.236 g. (M/100)
$MgSO_4$	0.0246 g. (M/1000)
$FeCl_3$	Trace
Starch	0.4 percent
Water	100 cc.
KH_2PO_4 or NaH_2PO_4	As indicated.

medium lacking potassium on the activity of the diastase. Determinations of the digestion of starch by Taka diastase in this medium show a very marked retardation apparently due to the concentrations of $Ca(H_2PO_4)_2$ and $MgSO_4$ present.

That the results in Table VIII do not necessarily indicate a connection between potassium and diastase secretion is also shown by the following experiment.

The time for digestion of a given quantity of starch in Czapek's medium,[9] with the carbon supplied as .4 percent of soluble starch and in media in which each of the elements generally accepted as essential for the fungi was replaced by some other element, was determined. The substitutions made were as follows:

Minus	nitrogen,	$NaNO_3$,	replaced	by	NaCl
“	potassium,	K_2HPO_4,	“	“	$CaHPO_4$
		KCl,	“	“	$Ca(NO_3)_2$
“	phosphorus,	K_2HPO_4,	“	“	K_2SO_4
“	magnesium,	$MgSO_4$,	“	“	K_2SO_4
“	sulphur,	$MgSO_4$,	“	“	$MgCl_2$
		$FeSO_4$,	“	“	$FeCl_2$
“	iron,	$FeSO_4$,	“	“	NaCl

Penicillium camembertii, *Aspergillus Oryzæ* (Alhbury) Cohn, *Mucor Rouxii* (Calm) Wehmer, and a species of *Fusarium* of the subulatum type, were used to inoculate these cultures. They were grown at room temperature and tested daily for starch by the method of Katz. As the culture medium showed complete disappearance of the starch the dry weight of the mycelium was determined.

It would appear from the data as summarized in Table X, which represent the averages of triplicate cultures, that nitrogen is the only element whose absence makes any considerable difference in the time required for digestion.

It is also noteworthy that these four fungi may be separated into two groups: the *Fusarium* sp. and *Mucor Rouxii*, which digest starch

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[9] The composition of this medium is

$NaNO_3$	2 g.
K_2HPO_4	1 g.
$MgSO_4$	.05 g.
$FeSO_4$	.001 g.
KCl	.05 g.
Water	100 cc.

TABLE X

Medium 60 Cc.	Fusarium sp.		Mucor Rouxii		Aspergillus Oryzae		Penicillium Camembertii	
	Time for Digestion (Days)	Dry Weight of Mycelium (Mg.)	Time for Digestion (Days)	Dry Weight of Mycelium (Mg.)	Time for Digestion (Days)	Dry Weight of Mycelium (Mg.)	Time for Digestion (Days)	Dry Weight of Mycelium (Mg.)
.4 percent starch....	249+ blue	—[10]	249+ blue	—[10]	7	.4	23	1.4
−K..........	11	18.7	121	—[10]	7	8.0	6	3.1
−N..........	249+ brown	10.6	249+ purple	—[10]	19	13.6	159+	12.3
−Mg........	9	37.2	74	—[10]	7	3.4	6	2.2
−P..........	11	41.9	42	34.1	5	3.4	6	5.3
−S..........	13	34.8	68	—[10]	10	20.4	6	16.8
−Fe.........	11	53.7	24	40.0	7	14.9	6	13.1
Full nutrient..	10	45.4	23	44.6	7	15.6	6	14.4

very slowly in the absence of nitrogen; and *Aspergillus Oryzæ* and *Penicillium camembertii*, which digest starch fairly rapidly in the absence of all nutrients.

It may also be noted that there is little correlation between the time required for digestion and the dry weight of the fungous mycelium. In the case of *Penicillium camembertii*, the same time (6 days) is required to digest approximately .24 g. of starch in the cultures lacking potassium, magnesium, phosphorus, sulphur, and iron, and in the full nutrient, but the dry weight of the mycelium in these cultures varies from 2.2 mg. to 16.8 mg. Somewhat similar results are shown by the data obtained with the three other fungi.

Discussion.—An examination of the complete data obtained in the experiments with nutrient cultures shows that again we have no evidence to demonstrate that potassium is concerned with diastase formation. With the exception of those cultures in which nitrogen was lacking, there are no marked differences in the times required for the starch digestion. It is significant, as shown in Table X, that a longer period is required for starch digestion by the fungus when grown in the culture medium lacking nitrogen only, than in the medium lacking all nutrients. It was found that the combination of salts used in the minus nitrogen medium decreases the time required for Taka diastase to change a given quantity of starch to the point at which it no longer colors with iodine. The longer period of time required for digestion in the minus nitrogen culture medium is, therefore, due to a decreased secretion of diastase. As was found with the single salts, the presence of salts decreases the secretion of diastase.

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[10] Dry weight not determined.

Summary

I. A method of determining diastatic action in solutions of soluble starch by the precipitation of the undigested starch and a part of the dextrins in acid alcohol is described.

II. The addition of the chlorides and the sulphates of potassium, sodium, calcium and magnesium, singly, to a solution of Merck's soluble starch in distilled water treated with carbon black, decreases the amount of starch digested by *Penicillium camembertii* when the salts are present in M/10,000 and M/1,000 concentrations.

III. The nitrates of potassium, sodium, calcium, and magnesium, when present singly in M/1,000, M/10,000, and, in the case of the nitrates of calcium and magnesium, in M/100,000 concentrations, in a solution of Merck's soluble starch in distilled water treated with carbon black, increase the amount of starch digested by *Penicillium camembertii*.

IV. The addition, singly, of the nitrates of potassium, sodium, calcium, and magnesium to a solution of Merck's soluble starch in distilled water treated with carbon black, decreases the amount of starch digested by *Penicillium camembertii* per unit of dry weight of mycelium when the salts are present in M/1,000, M/10,000 and M/100,000 concentrations.

V. The dihydrogen phosphates of sodium and potassium, with the exception of M/1,000 KH_2PO_4, do not decrease the digestion of starch when present in M/1,000, M/10,000 and M/100,000 concentrations.

VI. Potassium salts inhibit the digestion of Merck's soluble starch in distilled water treated with carbon black more than do sodium salts.

VII. A marked difference is noted between the speed with which *Aspergillus Oryzæ* and *Penicillium camembertii* digest soluble starch in the absence of all added nutrients, and the rate of digestion by *Mucor Rouxii* and *Fusarium* sp.

VIII. No evidence was found to connect potassium and calcium with diastase formation.

IX. Nitrogen may bear an intimate relation to the formation of diastase by *Penicillium camembertii*.

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THE ARCHEGONIUM AND SPOROPHYTE OF TREUBIA INSIGNIS GOEBEL

DOUGLAS HOUGHTON CAMPBELL

One of the largest and most interesting liverworts is *Treubia insignis* discovered by Goebel in western Java, and named by him for the late distinguished director of the famous botanical gardens at Buitenzorg in Java, Dr. Melchior Treub.

The plant was collected near Tjibodas on Mt. Gedeh, a volcano in western Java, and it has since been found repeatedly by various botanists in this neighborhood. Schiffner[1] gives this as the only known habitat, but later collectors have found the plant (or a closely related species) in several widely separated regions. Goebel himself collected a Treubia in New Zealand, and it has been reported from Tasmania, Tahiti, Samoa and Patagonia.

"Stephani"[2] recognizes two species, *T. insignis*, from Java and Tahiti, and *T. bracteata* from Samoa. Sterile material only has been found in this latter species. *T. bracteata* has recently been reported from Tasmania,[3] and it is not unlikely that the New Zealand species is the same.

In May, 1913, the writer collected a single specimen of Treubia on Mt. Banajao in Luzon, Philippine Islands. The specimen was sterile, but except for its somewhat smaller size, it seemed to be identical with material collected in Java.

During a visit to Java in 1906 the writer secured a large amount of material near Tjibodas, where the plant was found growing in some places in great profusion on the ground and on rotten logs. Only a few plants with sporogonia could be found, but a number of plants

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[1] Die Hepaticae der Flora von Buitenzorg. Leiden. 1900.

[2] Stephani, F. Species Hepaticarum. Mém. Herb. Boiss., **16**. 1900.

[3] Rodway, L. Notes on *Treubia insignis* Goebel. Papers Proc. Roy. Soc. Tasmania.

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bearing archegonia were secured, and many plants with the characteristic gemmae. No antheridial plants were found in the material collected, and it is probable that the rarity of male plants accounts for the small number of fertilized female plants.

Treubia not only is one of the largest liverworts, but it shows a number of interesting structural features which have been pretty thoroughly investigated by Goebel[4] and more recently by Grün.[5]

It has a thick fleshy midrib or axis, and develops two rows of very large and distinct leaves of the "succubous" type, *i. e.*, the hinder margin of a leaf overlaps the forward margin of the next older leaf (fig. 1, *A*). At the base of each leaf, at its junction with the axis, a conspicuous appendage or scale (1) is formed upon the dorsal side, and this scale in the fertile plants covers the groups of archegonia and antheridia. These scales are connected by ridges which form a zigzag line along the center of the axis. In many plants there are produced groups of gemmae in the same position as the sexual organs. No amphigastria or ventral appendages can be seen.

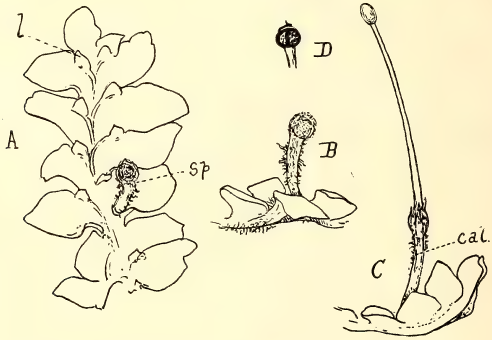

FIG. 1. *A*. Plant of *Treubia insignis* Goebel, × 1½. *sp*, sporophyte, still enclosed in the calyptra; *l*, dorsal scale. *B*. The sporophyte seen from the side. *C*. Plant with mature sporophyte, × 1½; *cal*, calyptra. *D*. An open capsule, × 3.

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[4] Goebel, K. Morphologische und biologische Studien IV. Ann. Jard. Bot. Buitenzorg 9. 1891.

[5] Grün, C. Monographische Studien an *Treubia insignis* Goebel. Flora 106. 1914.

The shoot branches monopodially, but neither Goebel nor Grün determined exactly the origin of the lateral branches, nor their relation, if any, to the leaves. The plant may reach a length of 16 cm. with an extreme breadth of 2.5 cm.

Although the general aspect of the plant is that of a very large acrogynous leafy liverwort, in the position of the archegonia and sporophyte, it is distinctly anacrogynous, *i. e.*, the apical cell of the shoot is not transformed into an archegonium. Unlike most of the Anacrogynae, *e. g.*, Pellia, Mörkia, Pallavicinia, etc., the archegonia do not arise in the median plane of the shoot but are formed in lateral groups subtended by the scales at the base of the leaves. Goebel compares their position to that in Fossombronia, where the archegonia are also lateral in position; but in the latter the archegonia are formed singly and not in groups, and instead of being protected by a distinct scale are covered only by the inrolled margin of the young leaf.

Goebel showed that the growth of the shoot is due to a three-sided pyramidal apical cell, very much like that of the typical Acrogynae, and his statement has been verified by Grün. As in most leafy liverworts the ventral face of the apical cell is smaller than the two dorsal lateral faces. From the latter, segments are cut off, each of which gives rise to a leaf, but no trace of leaves (amphigastria) are produced from the ventral segments.

The leaves are very large, and, except for the extreme margin, are composed of several layers of cells. From the ventral side of the leaf is developed a wing-like outgrowth which extends for a short distance along the ventral surface of the axis. On this wing are developed many mucilage-secreting papillae which exude great quantities of a colorless slime. Goebel suggests that the abundance of these secreting organs on the leaves accounts for the absence of the secretory hairs or scales that are so commonly found on the ventral surface of the apical region in most thallose liverworts. These secretory papillae may be single cells, or they may be stalked organs. The mucilaginous secretion fills a shallow furrow which occupies the ventral side of the midrib, and within this furrow are numerous short rhizoids. Goebel found in some of the cells of the thallus oil-bodies much like those occurring in the Marchantiales. Similar, but smaller oil-bodies occur also in many other liverworts.

There is always present an endophytic fungus which is very abundant in the ventral region of the shoot, and mainly confined to a definite zone just above the shallow furrow already referred to. The writer made no special study of this mycorrhiza which is evidently very similar to that found in a number of other liverworts, as well as in the gametophytes of a good many ferns, notably the Marattiaceae.

Goebel discusses at some length the nature of the dorsal scales which protect the reproductive organs but does not come to a definite conclusion. He thinks they may be considered either as independent structures, or as part of the leaf. According to Goebel's account, which has been confirmed by Grün, the young segment of the apical cell, from which this leaf arises, shows, when seen from the surface, three cells, of which two give rise to the leaf, and one—that nearest the midrib—to this scale. The relation of the leaf and scale is therefore the same as that of the two lobes found in the leaves of so many acrogynous liverworts, and it seems to the writer that this is probably the simplest interpretation of the case in Treubia.

Treubia is in several respects much like Fossombronia. This is true of the origin of the leaves, and in the position of the archegonia. Fossombronia, like Treubia, usually is infested by a mycorrhizal fungus—at least this is true for *F. longiseta*,[6] which also shows small oil-bodies in some of the leaf-cells. These, however, are much less conspicuous than the large oil-bodies of Treubia. Fossombronia differs from Treubia in the form of the apical cell,[7] which is of the two-sided type found in moss Anacrogynae. One of Humphrey's figures of *F. longiseta*,[8] suggests the possible occurrence of a three-sided apical cell in this species.

Another liverwort which has the same type of apical cell as Treubia is Noteroclada (Androcryphia), and Schiffner,[9] who has studied this rare liverwort, concludes that it is nearly related to Treubia with which it agrees not only in the form of the apical cell, but also in its leaf structure. Schiffner thinks that Noteroclada is also related to Fossombronia, with which it is connected by Petalophyllum.

THE ARCHEGONIUM

Goebel made no special study of the archegonium, but Grün has given a pretty satisfactory account of its most important features, which have been confirmed for the most part by the writer's own observations.

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[6] Humphrey, H. B. The Development of *Fossombronia longiseta*. Annals of Botany, 20. 1906.

[7] Leitgeb, H. Untersuchungen über die Lebermcose, Heft III. Jena. 1877.

[8] Humphrey. L. c. Text-fig. 4, *H.*

[9] Schiffner. Zur Morphologie von Noteroclada. Österr. Bot. Zeitschr. **61.** 1911.

The archegonia occur in groups, sometimes as many as a dozen together. As we have already seen, they are laterally placed, and each group is in the axil of one of the dorsal scales, which completely covers it.

The youngest archegonia (fig. 2, *A*) are hemispherical cells. The first division may be a horizontal one, cutting off a short stalk-cell—or it may be strongly oblique. In the latter case the first wall is quickly followed by two similar ones, which intersect so that a central (terminal) cell is formed which appears triangular in longitudinal

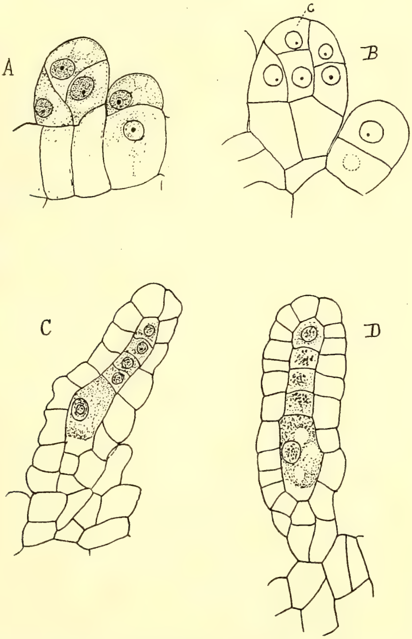

FIG. 2. *A*. Two very young archegonia, × 550. *B*. A somewhat older stage, showing the cap-cell, *c*, × 550. *C*, *D*. Older stages, showing four neck canal-cells, × 335.

section. Where a stalk cell is first cut off, this is followed by three nearly vertical intersecting walls in the terminal cell. The central cell in this case is truncate below (fig. 2, *B*) instead of pointed. In either case the next division is usually at least a transverse wall in the central cell, cutting off a cap-cell (fig. 2, *B*, *c*), which finally is divided into four by intersecting vertical walls.

The subsequent divisions in the archegonium follow the usual course; *i. e.*, a series of transverse divisions, in all but the cap-cell,

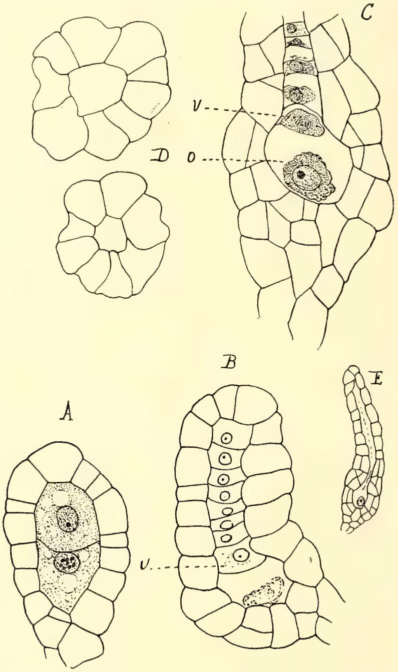

FIG. 3. *A*. Young archegonium, showing the separation of the central cell and the primary neck-canal cell, × 550. *B*. An older archegonium with seven neck-canal cells, and ventral canal-cell, *v*. *C*. The venter of a nearly mature archegonium. *o*, egg; *v*, ventral canal-cell, × 550. *D*. Two cross sections of the archegonium. Neck, × 550. *E*. Adult archegonium, × about 100.

separates the lower or ventral region from the neck; and in the three primary peripheral cells of the neck, a longitudinal division inaugurates six rows of neck-cells. In the older archegonium of Treubia, however, other longitudinal walls may appear, so that a cross-section of the neck, especially in its lower part, shows sometimes as many as nine peripheral cells, while most of the Jungermanniales have but five. As a result of this increased number there is not a clearly marked line between the venter and the base of the neck (fig. 3, *C*, *D*).

Figure 2, *C*, *D* show longitudinal sections of two young archegonia in which the central cell of the venter is still undivided, and the primary neck canal-cell has divided twice. As in other liverworts, there is later cut off from the central cell, the ventral canal-cell (fig. 3, *C*, *v*), and there is a further division of the neck canal-cells. None of the specimens examined showed more than eight neck canal-cells, but Grün gives a figure showing sixteen, which he says is the normal number for the fully developed archegonium.

The cells forming the wall of the venter undergo periclinal divisions, so that at maturity the egg is surrounded by a double layer of cells.

The number of archegonia in a group, in the specimens examined by the writer, was about a dozen. Mingled with the archegonia are numerous paraphyses ("Paraphylls"), which may be either simple cell-rows, or more or less expanded and branched scales. The marginal cells of these scales are often secretory organs, exuding a mucilaginous matter like that developed from the mucilage papillae on the lower side of the leaves.

The Embryo

Grün has described somewhat at length the structure of the older sporophyte, but he was unable to get the earliest stages. The account here given is far from complete, owing to the limited amount of material that was available; but it is hoped that it will be sufficient to make clear the most important points in the early history of the sporophyte.

The earliest divisions were not seen, but it is pretty certain that they are transverse as in all other Jungermanniales that have been investigated. It is also reasonably certain that the lowermost segment (or segments?) are devoted to the formation of the conspicuous haustorium which is a marked feature of the young embryo. All of the structures of the older sporophyte, foot, seta and capsule, are presumably developed from the terminal cells of the young embryo, as they are in other similar forms, *e. g.*, Podomitrium, Pallavicinia, etc.

Figure 4, *A*, shows a young sporophyte in which the basal region constitutes a haustorium made up of large cells. The embryo at this stage closely resembles that of certain species of Pallavicinia,[10] but the haustorial cells are relatively smaller and more numerous. The cells of the haustorium have very much larger nuclei than those of the upper part of the embryo (fig. 4, *B*, *C*). In the later stages of development these haustorial cells become very much compressed by the rapid growth of the foot of the young sporophyte, which evidently replaces them as an organ of absorption. A similar condition was noted by the writer in Podomitrium.[11]

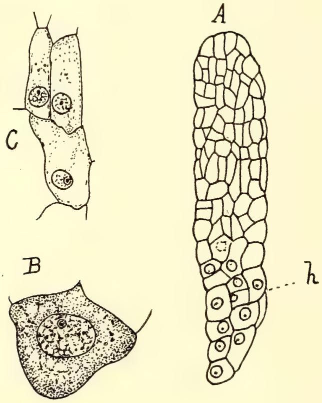

FIG. 4. *A*. Young sporophyte showing the large haustorium, *h*, × 84. *B*. A single cell of the haustorium, showing the large nucleus, × 375. *C*. Cells from the upper part of the sporophyte, × 375.

As the sporophyte grows the lower portion enlarges slightly to form the rather indefinite foot (fig. 5, *C*, *f*) while the terminal region, which is only slightly broader than the intermediate portion (seta), begins to show the first evidences of a differentiation of sporogenous tissue.

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[10] Campbell, D. H. and Williams, F. A Morphological Study of Some Members of the Genus Pallavicinia. Stanford University. 1914.

[11] Campbell, D. H. The Morphology and Systematic Position of Podomitrium. Amer. Journ. Bot. 2, 199. 1915.

In the development of the primary sporogenous tissue Treubia resembles most nearly, of the forms that have been investigated, Podomitrium. As in the latter the young sporogenous tissue is very vaguely defined, and it is quite impossible to determine exactly its extent (fig. 5, *A*). In this respect Treubia differs from Pallavicinia, Aneura or Fossombronia, where the limits of the young archesporium are much more definite, this being especially marked in Aneura and Fossombronia, where the archesporium is recognizable at a very early stage of development.

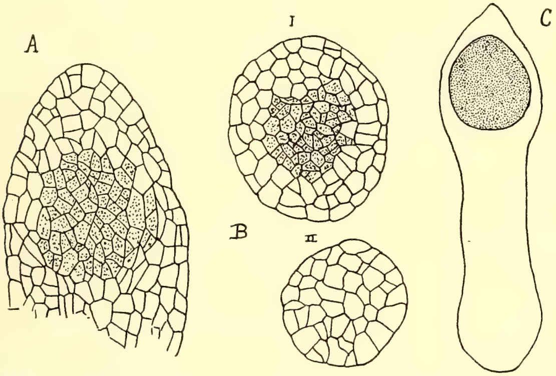

FIG. 5. *A*. Upper part of young sporophyte, × 84; the sporogenous tissue is shaded. *B*. Two cross-sections of a sporophyte of about the same age as that shown in *A*. *C*. Longitudinal section of an older sporophyte, × 27.

The sporogenous region is bounded by several layers of sterile tissue, which form the wall of the capsule. This is about three cells thick at the sides, but at the apex of the capsule there may be as many as eight, and a conspicuous beak is produced as in Pallavicinia and Podomitrium, and to a lesser degree in Calycularia (fig. 6, *A*). In this respect Treubia also differs from Fossombronia and Aneura where the apical part of the capsule wall (aside from the elaterophore) is of the same thickness as the lateral wall.

The sporogenous region becomes more clearly defined as cell-division proceeds, and there is soon to be made out a distinction between spore mother-cells and elaters. No definite relation could be made out between these. The elaters occur either singly or in small groups irregularly distributed among the very much more numerous young spore mother cells (fig. 6, *B*). A few elaters could often be found radiating from the base of the capsule, but there was nothing which could be described as a definite elaterophore. The elaters are less numerous than is usual among liverworts, but they finally attain a length which probably exceeds that of any other known form.

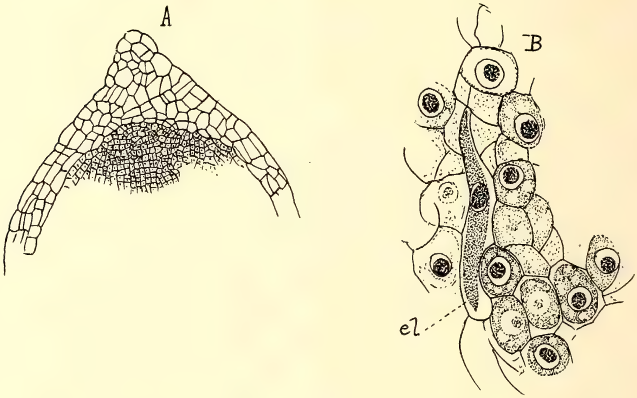

FIG. 6. *A*. Upper part of a young sporophyte showing the beak-like prominence at the apex, × 60. *B*. The sporogenous tissue from an older sporophyte showing a young elater (*el*) and the young spore mother-cells, × 400.

Grün[12] states that the rounding off of the spore mother-cells and the spaces between them which are seen in the later stages are partly due to the disintegration of some of the sporogenous cells; a careful examination of the writer's preparations of these stages, which were well fixed and stained, showed no evidence of the breaking down of any of the cells, and it seems practically certain that the separation of the elaters and the rounding off of the spore mother-cells can be perfectly explained as the result of a partial dissolution of the cell-walls, together with the rapid enlargement of the capsule in the later stages of development, which is not accompanied by a corresponding increase in the size of the spore mother-cells.

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[12] L. c., p. 372.

Lack of material made it impossible to study the spore division, as no stages were found between that shown in figure 6, *B*, which shows the young spore mother-cells before the final divisions had begun, and nearly ripe spores. The material was fixed in acetic alcohol, so that the finer details of the nuclear structures were not very satisfactorily shown. The nuclear contents were often contracted, especially in the spore mother-cells; but whether this was a normal synapsis, or, what is more likely, the result of imperfect fixation, was not determined.

Grün succeeded in finding the dividing spore mother-cells, although not a sufficient number of stages to make out all the details. He found no indication of centrosomes such as Farmer[13] describes for *Pallavicinia decipiens*, and some indications of which were found by the writer in *Calycularia radiculosa*. Grün found sixteen chromosomes in the dividing cells of the sporogenous tissue before the final divisions of the spore mother-cells, but it is not clear just where the reduction division occurs. To judge from his account and figures, it seems that in *Treubia insignis*, as in *Pallavicinia decipiens* and *Calycularia radiculosa*, there is a "quadripolar" spindle, and not two successive bi-polar spindles such as usually are found in spore division.

The calyptra (fig. 1, *C*, *cal.*) enclosing the developing sporophyte is very large in Treubia, where it may reach a length of nearly 1.5 cm. and in also very massive. The surface develops scale-like outgrowths which give it a shaggy appearance, and among the scales may be seen the remains of the unfertilized archegonia. The fully developed sporophyte has a seta about 35 mm. in length and the ovoid capsule is about 2.5 mm. in length (fig. 1, *C*). The capsule dehisces by four somewhat irregular valves (fig. 1, *D*).

Andreas[14] has given a fairly complete account of the structure of the wall of the mature capsule, and Grün has supplemented this by a careful study of the development of the wall in the later stages.

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[13] Farmer, J. B. On *Pallavicinia decipiens*. Annals of Botany, 8. 1904.

[14] Andreas, J. Über den Bau der Wand und die Öffnungsweise des Lebermoossporogons. Flora **86**. 1899.

Except for the apex, which as we have seen has a conspicuous beak formed of several (8–10) layers of cells, the capsule wall is usually composed of three layers. In the earlier stages these layers are composed of uniform cells, but as development proceeds the two inner layers undergo more or less numerous divisions while the cells of the superficial layer remain undivided and increase much in size as the capsule enlarges. On the walls of the two inner cell-layers characteristic thickenings are formed while the walls of the superficial cells undergo little change. The thickenings on the walls of the inner cells are of various kinds—ridges, complete rings, half-rings, and spirals. Grün states that these cells contain chlorophyll and starch granules. The ripe capsule is ovoid in outline, and not globular, as Andreas states. It opens by four short and somewhat irregular valves. Grün examined the ripe spores and elaters. The former have reticulate thickenings upon the surface, and resemble the spores of certain species of Pallavicinia. They measure from 20 to 25 μ in diameter. The elaters reach the extraordinary length of 1,250 μ.

Conclusion

Most writers agree that Treubia has much in common with the acrogynous leafy liverworts and in a sense connects them with the typical anacrogynous forms. Among the latter, the genera Fossombronia, Ptalophyllum and Noteroclada are most nearly related to Treubia. Cavers[15] in his recent summary of the Hepaticae considers these to have been derived from Pellia-like ancestors, but he looks upon Fossombronia as most nearly related to the Acrogynae.

It seems more likely that Treubia is nearer to the Acrogynae than is Fossombronia. This is true of the character of the leaves, the apical cell, and the groups of archegonia. It is by no means impossible that the dorsal scales may be the homologue of the dorsal lobe of such leafy liverworts as show a bilobed leaf, *e. g.*, Madotheca, Frullania, etc. Schiffner,[16] who has studied Noteroclada concludes that it is closely related to Treubia and must be considered as the end of a series of which Fossombronia is a lower member.

Fossombronia differs a good deal from the Pellia type, and is in some respects much more like Geothallus, which in turn is unmistakably closely related to Sphaerocarpus. It is possible that the Fossombronia line (including Petalophyllum, Noteroclada and Treubia) is a direct development of the Sphaerocarpus type and is not closely related to the Pellia line (Codoniaceae). Moreover it is not unlikely that from the Fossombronia line, the Acrogynae (or part of them) have originated. Should this hypothesis be correct, it would necessitate the removal of the series of forms—Fossombronia, Treubia—from the Codoniaceae and their association with the Sphaerocarpales.

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[15] Cavers, F. The Inter-relationships of the Bryophyta. New Phytologist Reprint, No. 4. Cambridge, 1911.

[16] L. c.

THE ORIENTATION OF PRIMARY TERRESTRIAL ROOTS WITH PARTICULAR REFERENCE TO THE MEDIUM IN WHICH THEY ARE GROWN

Richard M. Holman

I. Introductory and Historical

The orientation of plant organs relative to external agencies has long been a subject of interest, not alone to botanists but to those without botanical knowledge as well. The fact that the trunks of the trees on a steep mountain slope orient themselves without reference to substratum and grow parallel to the direction in which the attraction of gravity operates illustrates no less forcibly than does the familiar bending of the stems of house plants toward the window from which they receive light the importance of external factors in directing the plant's growth. Similar phenomena are not uncommon among animals, although, aside from our own dependence upon gravity for the orientation of our bodies, there are about us fewer examples which are obvious to the untrained observer, of the directive effect of gravity, light and other external factors upon animals. There are, however, among those animals which, like most plants, remain attached during all or a part of their existence, many cases in which the orientation of the organism is dependent upon gravity, one-sided illumination or other external agencies acting in a definite direction. Unattached and motile animals can, in many cases, be shown to have the direction of their movements definitely determined by these and other external factors. Plants offer, however, more favorable material for the study of the directive influence of these agencies which are not diffuse in their application to the organism but which operate or can be caused to operate in a constant direction. The subterranean parts of the plant as well as the structures above ground are under the influence of various agencies which affect the direction of their growth. Chief among these is gravity, and the terrestrial root furnishes a particularly favorable object for the study of the directive influence of gravity. More investigation has probably been devoted to the study of the geotropism of roots than to any other subject related to the irritability of any single organ of the plant. Nevertheless, a number of problems in this connection are still without satisfactory solution. The influence of the medium upon the orientation of the terrestrial root is a problem which has received only slight attention from plant physiologists and it is to this problem that the present paper is largely devoted. The investigation was prompted by the following questions:

Why do primary roots, placed horizontally or directed obliquely upward in air, exhibit, after a growth of one or two days, a very flat curvature such that the growing region forms a considerable angle with the perpendicular, whereas, in earth, roots similarly placed curve acutely and arrive at the normal perpendicular position? Why do these roots in air frequently fail to bend to the perpendicular but grow for days in a direction oblique to the direction of the stimulus of gravity, while roots in earth always return to the perpendicular when displaced therefrom?

The problem was suggested to me by Professor Wilhelm Pfeffer while I was a student in the Leipzig Botanical Institute. I wish to express my very sincere thanks to Professor Pfeffer for his constant interest and encouragement in my work while I was a student in his institute. The greater part of the work was done in Leipzig, but certain experiments were made at the botanical laboratory of the University of California. I am particularly indebted to Dr. William A. Setchell, professor of botany at the University of California, for his kindness in securing for me an excellent centrifugal apparatus. Privatdocent Johannes Buder, of the Leipzig Institute, and Dr. T. H. Goodspeed, of the Department of Botany of the University of California, also furnished advice and assistance.

As far as I have been able to determine, the first reference to the difference in behavior, relative to gravity, of roots growing in air and earth is to be found in Hofmeister's paper (1869, S. 35, ff.) in defense of his conception of the "mechanics of the penetration of the root into the soil" which had been so ably attacked by Frank. Hofmeister explained the difference in curvature of roots in air and soil as resulting from an earlier loosening of the cells of the root cap when the root was constantly wet, as he believed it to be when growing in soil, than when the root was growing in air, where, even if occasionally wetted, it was not constantly in contact with liquid water. In the latter case he supposed so little of the "plastic" root tip to be free from the encircling root cap that the root curved only very slowly while the shorter root cap of the roots growing in earth did not restrict the curvature of the "plastic" portion of the root to any extent. The "plasticity" of the root tip and the rigidity of the root cap, assumptions necessary to Hofmeister's explanation, are no longer tenable but, quite aside from that fact, the similarity of the curvatures executed by roots growing in water to the curvatures of roots in air is quite sufficient evidence of the incorrectness of Hofmeister's explanation.

Sachs (1874, S. 444–447) also gave some attention to this problem and one section of his paper "Ueber das Wachsthum der Haupt- und Nebenwurzeln," bears the heading "Verschiedenheit der Krümmung in Luft, Wasser, Sand und Erde" and deals with the subject with which we are concerned. In another part (S. 455–456) of the same paper Sachs suggests certain reasons for this difference of behavior. He attributes the differences in the course of the curvature of roots in earth or sand on the one hand and water or moist air on the other to four factors:

1. A more rapid growth of the under than of the upper side of the curved roots in air or water. This, he believed, caused the flattening of the curvature.

2. The resistance which the earth or sand offers to change in the form of the curvature.

3. A loss by the forward part of the root of the ability to curve further.

4. The assistance supplied to the geotropic curvature of roots in earth or sand by a positive thigmotropic reaction resulting from the greater friction of the lower than of the upper side of such geotropically curving roots in their passage through the soil or sand, *i. e.*, against the soil or sand particles.

Elfving (1880, S. 32, ff.) performed experiments in which he compared the curvature in air of inverted roots and of roots placed on a centrifuge and subjected to a stimulus of 50 g. for 24 hours, the tips being at the beginning directed toward the axis of rotation. Observing a more complete curvature of the latter than of the former he concluded that roots in earth probably possess a greater sensibility for the stimulus of gravity than do those in air.

Němec (1901, *a*, S. 88–96 and 1901, *b*, S. 310–313) in two papers, devoted primarily to an effort to substantiate the statolith theory, reported experiments and observations on roots diverted from the normal position while growing in moist air. He subscribed to Sachs's belief that contact stimulation of roots growing in earth assists and renders more acute the downward curvature. He proposed the following two explanations for the failure of air and water grown roots to reach the vertical and for their subsequent growth straight ahead in an oblique position:

1. That through long-continued geotropic stimulation the root becomes "tired" and the autotropism of the organ gains the upper hand. This would mean that a position of rest is reached by such a root before it has curved clear to the perpendicular, except when some other stimulus such as contact assists the geotropic stimulus.
2. That during their reaction the roots undergo such a change of geotonus that they become plagiotropic like secondary roots, the perpendicular position of rest being now replaced by a new position of rest to which the root tends to return after diversion therefrom.

It is the latter of these explanations which Němec favors. In the two papers cited and in a third (1904, S. 45–51) appearing three years later, he reports the results of experiments in support of his theory concerning the change of orthotropic roots to a plagiotropic condition. In these papers, he also reports his observations on the changes in the supposed perceptive apparatus, changes which take place simultaneously with the taking on of the supposed plagiotropic condition.

More detail and critical consideration of these conclusions of Němec as well as of the contributions of Sachs and Elfving will be reserved for the main body of this paper.

II. Methods

The seedlings employed in this investigation were grown from seeds soaked for twenty-four hours in water, rinsed thoroughly and planted in uniformly moistened sawdust which had been well rubbed between the hands and which had been placed in pots without compression. A thin layer of moist sawdust was placed over the seeds and they were then permitted to germinate at a temperature of from 18° to 20° C. In some of my experiments seedlings were grown for many days and even weeks in moist sawdust and on that account it is not out of place to comment upon the quality of the sawdust used. It was very uniform, absorbed a large amount of water and yet packed very slightly even when it had stood for many days. Roots which had grown in this medium to as great a length as 40 cm. appeared normal in every way.

Two methods were employed to assure a sufficient supply of water to the seedlings when the roots were kept in air during the course of an experiment. In some cases the cotyledons and the older parts of the roots were wrapped in wet filter paper or cotton while in other cases, the entire seedling, with the exception of the terminal 1 to 2 cm. of the root, was planted in moist soil in a pot through holes in the wall of which the ends of the roots projected. At the place where they passed through the wall of the pot, the roots were wrapped in narrow strips of moist filter paper. All roots cultivated in air were frequently sprayed and the atmosphere around them was kept as nearly saturated as possible by lining the bell jars or other receptacles in which the cultures were kept with wet filter paper and in addition by maintaining as constant a temperature as possible.

When the object of an experiment required that the position of a root in air be changed after it had been under observation for some time, the seedlings were mounted on corks, each of which was cemented somewhat eccentrically in a shallow crystallizing dish. These dishes were closed with glass plates held in place by wire spring clamps and, as far as was possible without interfering with the observation of the roots, were lined with wet filter paper. The crystallizing dishes were kept on edge throughout the experiment. This was easily accomplished by fitting them into open rings of galvanized metal fastened to blocks of wood. During the intervals between observations the cultures were placed under bell jars or in zinc boxes lined with wet filter paper.

In every experiment, unless a statement to the contrary is specifically made, the seedlings were kept in darkness between observations and when they were inspected care was taken not to expose them to the light for any considerable length of time. In every experiment in which the behavior of two sets of roots under different conditions was compared, the roots of the two series were carefully matched as to length and other visible qualities before they were used; that is to say, each root in one series was of the same length and of approximately the same diameter and general appearance as the corresponding root in the other series.

Seedlings whose roots were to be under observation while growing in soil, sand, sawdust or other more or less consistent mediums were planted in Sachs's boxes or in similar boxes with parallel glass walls. When the experiment involved the complete inversion of a culture or a turning of it through 90°, the boxes with parallel walls were the more convenient. Boxes which were to be turned on edge or inverted during an experiment were provided with lids of paraffined heavy cardboard, perforated and held in place by wire or by means of plaster of Paris.

When, in recording the results of an experiment, it was only of importance to know the position of the terminal portion of the root relative to the vertical, the angle was measured by means of a paper protractor pasted upon a semicircular piece of zinc plate and provided with a plumb and a thread so that when the straight edge of the protractor was placed parallel with the root the thread indicated the angle of the root with the perpendicular. After a little practice the angle could be determined with an error not to exceed two degrees. When it was necessary to detect slight changes in the position of the root tip or to compare the form of the curvature of the root at intervals, a drawing camera which I have elsewhere described (Holman, 1915) was employed. This camera had the distinct advantage that, by its use, drawings could be made of the roots without changing their position relative to gravity. The drawings were made on parchment paper and those made at different times could be superimposed so that when viewed by transmitted light the whole course of the root's curvature could be followed.

Throughout this paper I have used the term "flattening" with reference to the increase in the radius of the geotropic curvature of a primary root in air which generally takes place after the geotropic curvature has reached a maximum. The word has been used in the same sense as Czapek, Simon and others have used the word "Ausgleich." It has been found convenient to employ the term "primary geotropic curvature" to designate the geotropic curvature of roots in air taking place before the beginning of the autotropic flattening as well as the geotropic curvature of any previously uncurved root regardless of the medium in which it executes the curvature. The curvature of roots subsequent to the primary geotropic curvature and to the flattening of the primary curvature has been designated in this paper as the "secondary geotropic curvature," regardless of the medium in which this secondary curvature takes place.

III. Cause of the Difference in Curvature of Main Roots Growing in Earth and in Air

As Sachs pointed out, there are two particulars in which the behavior of primary roots in air which are considerably diverted from their normal position differs from the behavior of such roots similarly placed in soil.

First, the roots in the more consistent medium do not flatten their geotropic curvature. They undergo no change in the form of their curvature after the perpendicular is reached. The roots in air on the contrary, as soon as the geotropic curvature has reached a maximum, flatten this curvature. Thus the terminal portion of the root comes to form a considerable angle with the perpendicular.

Second, the roots in air after they have undergone geotropic curvature and flattening of that curvature may elongate in an oblique direction for several days and during this time only very slight further curvature, if any, generally takes place. On the other hand, roots in soil which have executed a geotropic curvature, if again diverted from the perpendicular and directed obliquely downward, again curve into the normal position.

Now, with reference to the first of these points, there is no reason to doubt that roots which have curved downward in earth and other firm media possess the same tendency to flatten their curvature as do roots in air. That the autotropic tendency is not absent from roots growing in media which do not permit of a change of the form of the root's curvature is indicated by the results of experiments performed by Simon (1912, S. 137 ff.) with roots which had curved under the influence of gravity while growing in moist sawdust. When removed from this medium and kept in air these roots gradually flattened their curvature. In some cases the flattening was so extensive as to decrease by 80 degrees the angle formed by the portions of the root above and below the original curvature. This, however, demonstrates with certainty only that growth in a relatively firm medium, even for several days after the geotropic curvature has been completed, does not prevent the flattening tendency being realized after such roots are brought into the air. As I have frequently observed, however, the roots of *Vicia faba* and *Lupinus albus* may immediately flatten their curvatures to a considerable extent when, after having executed a geotropic curvature in loose sawdust, they are carefully freed from the surrounding medium. As a result of this immediate autotropic flattening the angle of the terminal portion of the root with the perpendicular was in my experiments sometimes increased by as much as 40 degrees. Table I shows the extent of this immediate flattening in the case of two roots taken at random from the large number observed. In this case moist sphagnum was the growth medium instead of sawdust but in the latter material the results were similar.

TABLE I

Roots of Seedlings of Vicia faba var. equina Placed Horizontally in Loose Moist Sphagnum

After 40 hours the curved portion was freed from the sphagnum.

Root Number	Original Length	Angle with Perpendicular After 40 Hours	Angle After Release	Region Involved in the Flattening
1	6.2 cm.	14°	40°	5.8 cm.
2	7.0 cm.	9°	52°	3.0 cm.

This immediate flattening or "springing" of the root after release from the relatively firm medium surrounding it is evidence that in such media the same changes in the curved region of the root take place which result in the flattening of the curvature in the case of roots in air. The resistance of soil prevents any change in the form of the root's curvature. There is nevertheless an autotropic reaction which, however, results only in a pressure being exerted by the root upon the material above it. No further explanation than the mechanical hindrance to change in the root's form is necessary, then, for the first of the two mentioned particulars in which the behavior of roots in air and in earth differ.

There is no such simple and obvious explanation for the second point of difference—*i. e.*, the fact that roots in air may grow almost straight ahead in an oblique position for several days while in earth they grow straight only when in the normal perpendicular position. According to Sachs's (1874, S. 456) explanation, roots in air undergo a lessening of their geotropic sensibility and later Elfving also concluded that there is a lessening of the geotropic sensibility of the roots in air. Němec (1901, *b*) advanced the idea that the roots in air undergo such a change in the perceptive apparatus that they become actually plagiotropic. In addition to these two hypotheses, there is a third possibility relative to the geotonus of the root, which is that the roots in air become geotropically neutral after they have performed a geotropic curvature and flattened that curvature.

Quite independent of any change in the geotonus of the root, there is another factor which may be conceived of as entirely responsible for the difference in behavior of roots in air and earth or as co-operating with a weakened orthogeotropism to bring about the difference in behavior. This factor is the assistance of the root in soil or other consistent medium in the execution of its reaction by reason of some property of the medium which does not alter the sensibility of the root to the stimulus of gravity. The assisting factor might be, as Sachs suggested, a positive thigmotropic reaction in the same direction as the geotropic reaction. On the other hand, it might be conceived of as acting more directly, without another tropism being concerned. Thus the physical properties of the medium might be such that the root would be assisted mechanically in the execution of a prompt and complete reaction. Numerous examples can be cited where the rapidity with which a body undergoes change in direction depends upon the resistance offered by the homogeneous material through which it is moving. Thus the rudder of a boat, although able to change the direction of the boat rapidly in water would, at the same rate of speed, be unable to cause any but a very slight change of direction if the boat were moving through air instead of water. In a medium offering still more resistance than water to the passage of a body through it the same steering device would cause a more rapid change of direction than in water. Such a case may be suggested as illustrative of the possibility that the physical properties of some media may assist the root in executing a prompt and acute curvature.

I shall first consider whether a change in the geotonus of the root growing in air does actually take place, and then whether the root in earth or similar media experiences assistance or reinforcement of its curvature.

Is there a permanent change in the geotonus of roots which have grown and curved geotropically in air?

It is important to determine whether or not the change of geotonus of roots in air, if such a change does take place, is a permanent one. In order to answer this question it is only necessary to transfer roots, which have curved and flattened their curvature in air, to earth, without changing their position relative to the perpendicular. Of a great number of roots of *Vicia faba* var. *major* and *V. f.* var. *equina* and of *Lupinus albus* so treated, all curved downward into the vertical when placed in earth. Roots were employed varying in length from 3 to 12 cm. Three of the longer roots did not curve downward at once into the normal position when placed in soil. They executed a more gradual curvature than the others, although they also finally reached the perpendicular. (Very frequently long roots, even when they have not been kept in air or undergone previous curvature, approach the perpendicular position only very slowly when placed obliquely downward in earth.) In spite of repeated comparisons of the rate of downward curvature of roots placed horizontally in earth directly after removal from the germinating bed with that of roots similarly placed in earth after geotropic curvature and flattening of this curvature in air, I have been unable to detect any difference in the rapidity of the downward curvature. These facts indicate that any change in the geotonus of the root which may take place in air is lost when the root is brought into earth. If, as Němec asserts, the root growing out of the perpendicular in air becomes plagiotropic, this plagiotropism is replaced by orthogeotropism when the root is placed in soil. Also, any considerable weakening or complete loss of geotropism which the roots in air experience disappears when the roots are placed in earth or other similar firm medium favorable to growth. Whatever change in the geotonus of roots may take place in air, there is no appreciable permanent change; for upon return to soil or sand or other firm and resistant medium such roots react just as do roots which have never grown in air.

Are roots which have performed a geotropic curvature in air more weakly geotropic while they remain in that medium than are roots in earth?

As I have already remarked, Sachs and Elfving assumed a weakening of the geotropism of roots in air which resulted in the roots discontinuing their curvature before the perpendicular was reached. Sachs's assumption was not based upon any specific experimental evidence. The only basis for Elfving's (1880, S. 32, ff.) conclusion was the results of experiments which he performed with seedlings of *Pisum sativum* rotated upon a centrifuge at such a rate that the roots were subjected to a stimulus 50 times that of gravity. The experiments were continued only for twenty-four hours and at the end of that period the roots upon the centrifuge, which at the beginning of the experiment had been placed with the tips directed toward the axis of rotation, were found to form a smaller angle with the radius of rotation than inverted control roots, also growing in air, formed with the perpendicular. Since those roots which in his experiments were subjected to the more intense stimulus had taken up, after twenty-four hours, a position more nearly parallel to the direction of the stimulating force than had the control roots, Elfving concluded that it was merely on account of weakened geotropism that roots in air under the stimulus of gravity often underwent no appreciable curvature when growing obliquely downward. When subjected to a centrifugal force of 50 times the intensity of gravity the roots in Elfving's experiments must have suffered considerably from water shortage and their growth rate must have been much below that of the control roots. This fact and the short duration of his experiments make it impossible for us to know whether or not the roots grown upon the centrifuge had completed the flattening of their curvatures at the time the observations were made. If Elfving's experiments had been continued for a longer period, and if observations of the growth of the roots had been made, it might have been clear that after the same increase in length the curvatures were approximately the same.

TABLE II

Two Series of Roots of Vicia faba var. equina from 4.5 *to* 6 *cm. Long, of which One Series* (*r*) *was Subjected to a Stimulus of* 4 × *g to* 8 × *g on a Centrifuge and the Other Series* (*c*) *Remained Stationary*

The roots remained in moist air throughout the experiment.

Root Number	Original Angle of Root with Radius or Perpendicular		Angle After 30½ Hours		Growth in 30½ Hours	
	r	*c*	*r*	*c*	*r*	*c*
1	68°	66°	14°	25°	3.0 cm.	2.4 cm.
2	78°	76°	13°	70°	3.6 cm.	2.7 cm.
3	70°	88°	9°	72°	3.6 cm.	3.0 cm.
4	76°	....	16°	....	3.2 cm.	......
Mean.......	73°	77°	13°	55.6°	3.35 cm.	2.7 cm.

On that account I performed a series of experiments to determine the actual effect of varying intensities of stimulus upon the curvature of roots in air. In some of the experiments the seedlings were rotated so that the stimulus was greater than that of gravity. In others the stimulus was less than that of gravity. Experiments were also performed in which roots in earth were subjected to a stimulus less than that of gravity. This was done in order to determine whether roots thus treated would, like roots in air while under the stimulus of gravity, grow straight ahead in a position oblique to the stimulating force. The centrifuge first employed was one designed for the lecture table and intended for rotation at relatively low speeds. Being without provision for self-lubrication and having a very small disc this centrifuge proved unsuitable for experiments which were to extend over a longer period than twenty-four to thirty hours at 20° C. Experiments with this apparatus which were continued for from twenty-four to thirty hours and in which a stimulus of from 4 to 10 g. was employed yielded, as is shown by Table II, results corresponding to those of Elfving's experiments.

TABLE III

Roots of Pisum sativum Subjected to a Stimulus of 15 × *g to* 19 × *g upon the Centrifuge* (*r*) *and Kept at Rest* (*c*)

Each series consisted of 5 roots.

Root Number	Original Length, Cm.		Original Angle with Radius or Perpendicular		Angle after 24 Hours		Angle after 45 Hours		Angle after 69 Hours		Angle after 93 Hours		Length after 93 Hours, Cm.	
	r	*c*	*r*	*c*	*r*	*c*	*r*	*c*	*r*	*c*	*r*	*c*	*r*	*c*
1	4	4.3	158°	134°	65°	115°	30°	95°	20°	67°	26°	51°	11	11.1
2	4.3	4.0	143°	130°	80°	95°	50°	80°	40°	65°	16°	61°	10.1	11.7
3	3.1	2.8	130°	123°	77°	94°	62°	90°	25°	69°	13°	56°	8.7	8.3
4	5.0	5.0	130°	127°	95°	112°	65°	88°	30°	60°	20°	48°	13.7	11.3
5	4.5	4.5	130°	155°	76°	104°	35°	91°	24°	75°	12°	63°	11.0	11.9
Mean...	4.2	4.1	138°	134°	79°	104°	48°	89°	28°	67°	17°	56°	10.9	10.9

Although in these experiments, owing to the relatively low rate of rotation and the care taken to provide sufficient water, the rate of growth of the centrifuged roots was not below that of the control roots, yet the experiments were not continued for a sufficient period. On that account I performed other experiments extending over a longer period. For this purpose a centrifuge was employed having a much larger disc than the one previously used and provided with self-oiling bearings. This apparatus, which was constructed by Mr. Arntzen, expert mechanician, civil engineering laboratory, University of California, was kept in motion by a 1/15 horse power induction motor with a speed of 1,800 revolutions per minute. Speed was reduced by means of an adjustable friction drive. The revolving disk was made of laminated wood and upon it were mounted the zinc lined, glass covered boxes in which the seedlings were fixed. Table III gives the results of one of a number of experiments with this apparatus in which the behavior of roots of seedlings upon the centrifuge was compared with that of roots at rest. The experiment to which Table III refers was performed with *Pisum sativum* but similar results were obtained with *Vicia faba major.*

In addition to these experiments, which show clearly that increase in the intensity of the stimulus above that of gravity results in a more complete reaction of roots in air, other experiments were performed in which roots subjected to a stimulus of only a fraction of the intensity of gravity were compared with roots under the normal stimulus. The results of one such experiment are given in Table IV. The growth of the two series of roots was, in consequence of the care exercised to secure sufficient water supply, not appreciably different.

TABLE IV

Roots of Vicia faba var. equina of which One Series (r) was Subjected to a Stimulus of $\frac{1}{4} \times g$ *and the Other Series (c) Remained at Rest*

Root Number	Original Angle with Radius or Perpendicular		Angle After 44 Hours	
	r	*c*	*r*	*c*
1	83°	87°	69°	43°
2	86°	92°	69°	34°
3	88°	94°	76°	65°
Mean...........	86°	91°	71°	47°

As is seen in this table, decrease in the intensity of the stimulus below that of gravity results in a lesser curvature of the roots than takes place under the stimulus of gravity. As we have shown, roots under a stimulus of $n \times g$ in air tend to react as do roots in soil under a stimulus of $1 \times g$. It is also important to determine whether or not roots when grown in soil and subjected to a stimulus of g/n tend to execute curvatures similar to those of roots in air under the normal stimulus. With this question in mind, I experimented with seedlings of *Vicia faba* var. *equina,* planted in soil in small Sachs's boxes which were mounted upon a motor driven clinostat in such a manner that the roots were parallel to the axis of rotation. When the rate of rotation was so rapid that the stimulus exceeded $(3 \times g)/100$ all the roots, during a growth of 2–4 cm., bent until the end of each coincided with a radius of rotation. The curvatures were not appreciably different from those executed by roots under the normal stimulus of gravity. When subjected to a stimulus of $(4 \times g)/1{,}000$ the roots, in a large proportion of cases, bent very gradually away from the axis of rotation. Then, after attaining an oblique position, they grew, in many cases, in an apparently straight line; in other cases in a curve of large radius. When similar roots were rotated at a rate only half as rapid, the stimulus being thus reduced from $(4 \times g)/1{,}000$ to $(1 \times g)/1{,}000$, they made no visible response. The behavior of roots planted in soil and subjected to stimuli of $(35 \times g)/1{,}000$ and $(4 \times g)/1{,}000$ is shown in Table V.

TABLE V

Roots of Vicia faba var. equina Grown in Soil and Subjected to Stimuli of Less Intensity than Gravity

All roots originally placed parallel to the centrifuge axis.

Root Number	Original Number	Angle After 2 Cm. Growth	Angle After 4 Cm. Growth	Angle After 6 Cm. Growth	Angle After 8 Cm. Growth	Stimulus
1	7.0	36	10	10	10	$4/1000 \times g$
2	9.1	46	46	38	—	
3	7.7	45	30	32	32	
4	9.5	90	46	36	36	
5	8.7	60	42	21	—	
6	5.9	53	52	—	—	
7	6.4	70	67	—	—	
8	7.8	40	6	—	—	
9	7.5	27	34	—	—	
1	7.0	0	0	—	—	$35/1000 \times g$
2	6.6	0	0	—	—	
3	7.7	23	0	—	—	
4	6.9	33	0	0	—	
5	7.6	29	7	0	—	
6	10.3	0	—	—	—	
7	8.2	16	0	0	0	
8	7.5	0	0	0	0	
9	5.9	19	6	4	—	

Note.—Dashes indicate that roots did not attain the length referred to.

These results show that by reducing the stimulus below that of gravity roots in earth can be caused to execute curvatures like those of roots in air under the normal stimulus of gravity.

Although all these centrifugal experiments, furnishing much more complete evidence than Elfving presented, show that changes in the intensity of the stimulus may exercise upon the permanent curvature of the root an effect similar to that exerted by the different media, air and earth, it by no means follows that reduced sensibility of the roots in air is the cause of the difference in behavior with which we are concerned. Some other factor may, in the case of roots in air and earth, be responsible for the differences in curvature. This factor might be the presence or absence of some agency which without affecting the sensibility of the root can assist in the execution of the reaction. Such experiments as those of Elfving, which I have extended yield no conclusive answer to the question which I have sought to answer in this section of the present paper. Elfving's conclusion is however in no way contradicted by the evidence which I have secured.

Do roots which have performed a geotropic curvature in air become plagiotropic?

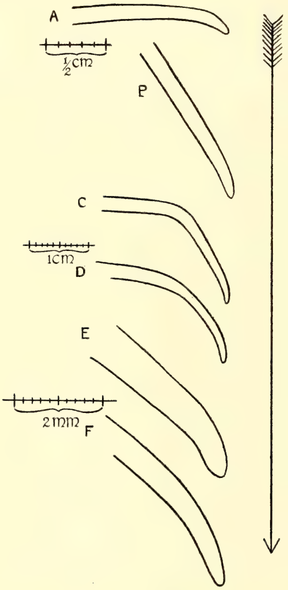

FIG. 1. Primary roots growing in air and showing the tip curvature. *A.* and *B.—Vicia faba* var. *equina; C.* and *D.—Lupinus albus; E.—Vicia faba* var. *equina* (this root had grown in moist air at 15° C. for 15 days); and *F.—Vicia sativa.*

Before discussing this question it is well to call attention to the curvature, often very acute, of the terminal 1 to 2 mm. of roots growing out of the normal position in air. Figure 1 illustrates the form of this curvature in the case of several roots used in my experiments.

This curvature of the extreme tip of the root first makes its appearance after a root has begun to flatten its primary geotropic curvature. From that time on, it persists as long as active growth continues, provided that the root is well supplied with water and that the elongating zone of the root does not lie in the perpendicular. As the root elongates the curvature is maintained at the very tip. When the cells of the curved tip pass into the phase of active elongation, the difference in the size of the cells of the upper and lower sides of the root is compensated so that the elongating zone is only slightly or not appreciably curved. In my preliminary experiments, I was struck by the constant occurrence and conspicuousness of this curvature of the extreme tip of the root. Later I found that Němec (1901, *a*, S. 93 ff.) had described it. It is remarkable that previous to the appearance of Němec's paper, no one had reported this peculiar behavior of the root tip. This may, perhaps, be accounted for by the failure of earlier investigators to maintain a sufficiently high moisture content of the air in which they cultivated roots. One of Sachs's figures (1874, Fig. 10*B*) shows this curvature of the tip quite unmistakably, but his paper makes no reference to it. A similar geotropic "counter curvature" of the tips of roots whose elongating zones were directed obliquely downward in consequence of a rheotropic reaction was described and figured by Berg (1899) and also mentioned by Juel (1900, S. 352) and Newcombe (1902, p. 269, ff.). The importance of this curvature of the tip in connection with the orientation of roots in different media will soon become apparent.

Now an orthogeotropic organ may be defined as one whose only position of rest is the perpendicular. Such an organ, when placed in any other position tends to curve until its sensitive zone is again in the perpendicular (cf. Jost, 1908, S. 530). Similarly a plagiogeotropic organ is not merely one that undergoes no curvature when placed in a horizontal or oblique position but is an organ which tends to bend back into the original position when removed from an oblique or horizontal position.[1]

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[1] Except when the organ concerned is placed with its long axis parallel to an earth radius. Czapek has shown that for secondary roots the perpendicular is a labile position of rest, regardless of whether the root tips point upward or downward.

Němec has put forward the hypothesis that "not too young" seedling roots of certain of the forms which I have used, when inverted in moist air become, after a time, plagiotropic. The evidence upon which he based this hypothesis was threefold:

First, that in some cases roots grow for many hours straight ahead while in an oblique position or in the horizontal and that the tip, although generally forming a smaller angle with the vertical than does the elongating zone, frequently fails to reach the perpendicular.

Second, that when the tips of the roots are brought into a perpendicular position pointing downward they bend upward again into an oblique position.

Third, that when the tips of such roots are displaced from the oblique position an accumulation of protoplasm takes place in the cells of the columella, this accumulation being in the same part of the perceptive cells at which a similar aggregation of protoplasm appears in corresponding cells of secondary roots which have been displaced from their normal position.

The third reason which Němec advanced in support of his belief that orthotropic roots may become plagiotropic cannot alone be considered conclusive. It is really significant only if the other points are definitely established.

My own numerous experiments with seedlings of *Vicia faba* var. *major*, *V. f.* var. *equina*, *Vicia sativa*, *Lupinus albus*, *Pisum sativum* and *Ervum lens* whose roots were surrounded by moist air and were placed in various positions between 30° and 180° from the normal position have convinced me that such roots do tend to attain a quite definite oblique position, varying from 30° to 60° from the perpendicular. Thereafter active curvature takes place very slowly if at all. The position of the terminal portion of the root may however change owing to passive bending of the region behind the zone of elongation by reason of the increasing length and weight of the younger part of the root. Roots of *Vicia faba* and *Lupinus albus* up to 2.5 or 3 cm. in length when placed horizontal in air generally bring the elongating region into this oblique position within 24 to 36 hours at a temperature of 18° to 20°. Older roots require a longer time. This oblique position in which the root frequently elongates without further active curvature is generally attained by an extensive geotropic curvature and subsequent autotropic flattening of the geotropic curvature. Often in the case of roots which are of considerable length before being employed for the experiment there is no rapid and extensive downward curvature which is later flattened. Instead the root curves gradually downward until a position is reached varying from 60° to 30° from the perpendicular. When the seedlings are so mounted at the beginning of an experiment that the roots are directed obliquely downward, the geotropic curvature is slight and in the great majority of cases completely flattened. The root, straight throughout except for the curvature of the extreme tip, then generally elongates without further appreciable active curvature. When roots after being taken from the germinating bed are directed obliquely upward a longer time is required for the attainment of the position in which they point obliquely downward than when the roots are placed horizontal. Old roots, especially those of *Vicia faba* and *Lupinus albus* when placed at an angle of 45° above the horizontal often fail to curve below the horizontal before growth comes to a standstill[2] and inverted roots of these species if longer than 3 cm. at the beginning of the experiment often fail even to reach the horizontal. This corresponds to the observations of Němec (1901, *a*, S. 94 ff. and 1901, *b*, S. 310) but his statements on the subject refer to roots which were under observation only for a period of from thirty-six to forty-eight hours after inversion. In many cases, though, roots which after two days are still directed obliquely upward or horizontally later bend gradually downward until they point obliquely down. There are frequently, however, cases in which inverted roots do not reach the horizontal even after as long a period as ninety-six hours. Roots of relatively large diameter such as those of *Vicia faba* and *Lupinus albus* in contrast to the slenderer roots of *Pisum sativum*, *Ervum lens*, *Vicia sativa* and *Phaseolus nanus* most frequently behave in this manner. In the case of roots placed in air at an angle more than 45° or 50° above the hori-

[2] Sachs (1874, S. 409) reported that roots of *Vicia faba*, after 3 to 4 days in air ceased growing entirely. By exercising all possible care, I have succeded in maintaining a relatively active growth for 12 to 13 days after the roots were brought into moist air. For example, of ten roots ranging in length from 2 to 13 centimeters placed horizontal in moist air and subject to a temperature of 17° to 18°, the following are the mean elongations for successive periods: 1st day—2.04 cm., 2d day—1.99 cm., 3d day—1.35 cm., 4th and 5th days (forty-two hours)—2.14 cm., 6th and 7th days (forty-eight hours)—2.24 cm., 8th and 9th days (fifty-three hours)—1.26 cm., 10th and 11th days (forty-eight hours)—.86 cm. One of the ten roots did not grow during the 10th and 11th day and seven others did not grow after the 11th day. Two of the ten roots, however, grew during the 12th and 13th day, respectively 1.4 and .8 cm. during the forty-eight-hour period.

zontal there is a slow downward curvature subsequent to the primary geotropic curvature and the flattening of that curvature. This secondary geotropic curvature, which may continue for as long as four to five days, appears to be due to a slight residue of the curvature of the extreme tip which is retained by the cells as they pass over into the phase of elongation. During this secondary geotropic curvature, the residue of the primary geotropic curvature of the root frequently undergoes further flattening. As a result of this decrease in the

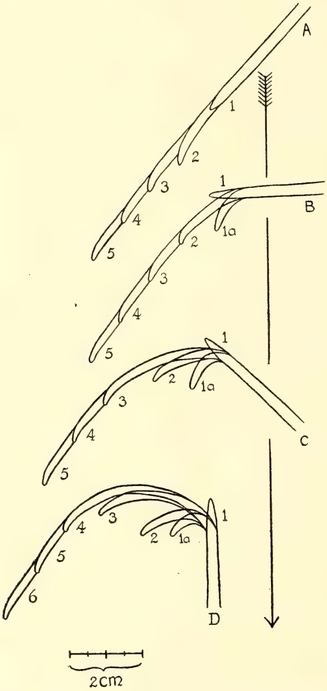

FIG. 2. Diagrams showing the behavior of roots of *Vicia faba* var. *equina* about 2 cm. long when placed in air at the angles shown at 1 in the diagrams.

curvature of parts of the root which have discontinued growth (cf. Simon, 1912, S. 133 ff.) the residue of the primary curvature may become so flat as to be indistinguishable from the very gradual secondary curvature. The failure of roots of *Vicia faba* and *Lupinus albus* which are longer than 4 or 5 cm. when inverted to bend below the horizontal is probably due to a slackening of the geotropic sensibility with length. Roots of large diameter when executing a curvature must maintain a greater difference in the size of the cells of the upper and lower sides than would slenderer roots. It is not unlikely that on that account a relatively greater intensity of geotropic stimulus is necessary to bring about corresponding reactions.

The behavior, described above, of roots variously placed in moist air is illustrated by the diagrams shown in figure 2. These diagrams were based upon a large series of camera drawings of roots of *Vicia faba* var. *equina* which were approximately 2 cm. long when displaced from the normal perpendicular position. Roots of *Lupinus albus* behaved in substantially the same manner.

Observation of roots of these species and of *Pisum sativum* and of other leguminous species indicates that, as illustrated in figure 2, roots in air when displaced from the normal position by more than 40° to 50° tend to take up a position in which the elongating region is directed obliquely downward, after which active curvature almost or entirely ceases. This fact does not however constitute conclusive evidence of the root having become plagiotropic. The failure of the root to undergo active curvature after this oblique position is reached can as well be explained by assuming that, after the primary curvature and its flattening have taken place, the relation between the autotropic and the geotropic impulse is altered in favor of the former and that a position of rest is then attained only after the inclination to the perpendicular is reached at which autotropism and geotropism are in equilibrium.

The crucial test for plagiotropism in the case of the roots in air is that which Němec (cf. p. 290 of this paper) states that he applied with positive results. I performed a number of experiments to determine whether the supposedly plagiotropic roots would actually bend upward into an oblique position when directed perpendicularly downward. Roots which had grown for one or two days in an oblique direction in air (as shown at 4 and 5 in figure 2) were placed with the tip pointing directly downward, the roots remaining in moist air.

The results of these experiments, in which the behavior of the roots was followed closely by means of enlarged tracings made with a camera, were entirely uniform. I found no case in which the root showed any tendency to bend upward and grow in an oblique direction. An account of one of the numerous experiments performed in this connection will be given here. Three seedlings of *Vicia faba* var. *equina*, whose roots were 2 to 2.3 cm. in length, were so placed that the roots were directed obliquely downward in moist air at an angle of 50° from the vertical. After sixty-four hours these roots were straight except for the curvature of the extreme tip and they were then so placed that the tips pointed directly downward. During the following seventy-seven hours, in spite of active growth, most careful observations made by means of enlarged camera drawings failed to show any trace of an upward curvature. No other change in the roots was noticeable except the increase in length and loss of the slight curvature of the root tips. Roots which were originally placed horizontally in air and which had reached the oblique position behaved in the same manner when they were turned until the tip pointed directly downward. Experiments with *Lupinus albus* yielded the same results as those with *Vicia faba* var. *equina*. Similar results were also obtained with roots which, after growth in the oblique position in air, were transferred to other media. Thus, of five roots of *Vicia faba* from 3.5 to 5.5 cm. long, placed at angles of from 30° to 50° in air, all, after having elongated from 4 to 5 cm. in the oblique position, grew straight ahead when the tips were directed downward and the roots surrounded by earth (in 2 cases) or loose moist sawdust (in 3 cases). Similar results were obtained with five roots of the same species which were similarly treated except that they were placed horizontal in air at the beginning of the experiment. After forty-eight hours they had reached an oblique position from 30° to 50° from the vertical and had elongated in that direction for some time, but when the tips of these roots were directed downward no upward curvature of the root took place. These results not only indicate that these roots had not become plagiotropic but also that they did not possess any induced dorsiventrality such as not infrequently occurs in the case of rhizomes and of branches of subaerial stems.

However in certain of Němec's (1901, *a*, S. 91, 92) experiments he obtained results which certainly seem to indicate an assumption of plagiotropism by the roots with which he experimented. He placed roots which were not "too young" in air with tips directed vertically upward. Then, after they had curved until the elongating region was approximately horizontal and the extreme tip acutely curved so that it pointed obliquely downward, he displaced the seedlings so that the tips of the roots were directed straight downward. At this time the roots were surrounded by moist sawdust. He subsequently observed an upward curvature of these roots opposite to the curvature of the tip at the time the roots were placed in the sawdust.

Upon repetition of Němec's experiments I found that a considerable number of the roots behaved as he reported.[3] In my experiments roots of *Vicia faba* var. *major* and var. *equina* and of *Lupinus albus* and *Pisum sativum* were employed. As in Němec's experiments, the roots frequently behaved in the same manner when they were inverted in loose moist sawdust at the very beginning of the experiment instead of being permitted to undergo curvature from the inverted position in air. After thirty-six hours these roots had so curved that the elongating zone was nearly horizontal and the curved tips pointed obliquely downward. When the cultures were then placed so that the root tips pointed directly downward, there followed in a varying proportion of the individuals an upward curvature of the tip which was always opposite to the original tip curvature. This upward curvature, which was in some cases very acute, was never completely fixed but was always somewhat flattened as the root grew. The curved region was also pushed forward from behind by the elongation of the region of the root behind it. Thus thirty-six to forty-eight hours after the upward curvature took place the root exhibited a flat curvature some distance below the position which the tip of the root had occupied. In earth the upward curvature was almost entirely suppressed and when it did appear it was only as a slight and transitory upward inclination of the tip. In the experiments with roots in earth the inverted root was kept in moist air until the elongating region had reached the horizontal and the tip pointed obliquely downward.

The roots in loose sawdust which bent upward did not, in my experiments, continue to grow in the oblique direction which they reached by reason of this upward curvature. Instead, in the course of subsequent elongation, they bent gradually downward until they reached the perpendicular. In this respect their behavior was like that of roots which had been allowed to grow for from thirty-six to forty-eight hours in air and which after attaining the oblique position of the elongating zone and curvature of the tip which I have described were transferred to loose sawdust without change in position. Although the rate of downward curvature varied with different individuals the final result was always the same, the attainment of a vertical position by the growing region of the root.

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[3] A full account of the conditions and course of Němec's experiments, which mine followed exactly in method, will be found in his paper already cited.

The striking behavior just described does not constitute conclusive evidence that the roots have become plagiotropic. In Němec's experiments and my own the apparently plagiotropic reactions were obtained even in the case of roots which had remained in loose moist sawdust throughout the experiments and yet eventually roots inverted in moist loose sawdust do reach the normal perpendicular position. There are other reasonable explanations for the upward curvature of roots which we have described than the assumption of a transitory plagiotropism by the root. As Němec (1904, S. 49–50) himself reported, roots with a distinct tip curvature, when rotated upon the clinostat often exhibit oscillating curvatures suggesting somewhat those which Baranetzky (1901) observed in the case of shoots. It may be that roots with sharply curved tips when released from one-sided stimulus by having the tips directed straight downward have the same tendency to oscillate as do the roots upon the clinostat. The first of these oscillations, if more intense that the succeeding ones might be partially fixed when the root was surrounded by sawdust. Later oscillations if considerably less intense than the first might be almost completely suppressed owing to the resistance offered by the medium. If this were the case no permanent upward curvature would result in the case of roots in air, a condition borne out by the experiments which I have reported above, and the slight tendency of roots in earth to execute the upward curvature would find its explanation in an almost complete suppression of even the first oscillation.[4]

Whether or not the preceding explanation is correct, it is possible to determine whether the upward curvature is a plagiogeotropic reaction by transferring roots after they have been inverted in air and have so curved that the elongating region is horizontal and the tip directed obliquely downward to loose moist sawdust in a receptacle mounted upon a clinostat. If the "upward" curvature appears also in the case of roots rotated upon the clinostat, we must assume that the curvature is of autotropic nature rather than, as Němec believed, the result of induced plagiotropism. I have performed a number of experiments with the object of thus determining the nature of the upward bending. In each of these experiments one series of roots was treated just as were those in Němec's experiments, *i. e.*, after inversion in air and curvature there such as has already been described, the roots were placed with the tips directed downward in loose moist sawdust, while a second series was similarly treated except that after being placed in the loose sawdust they were rotated upon the clinostat. In one of these experiments seedlings of *Lupinus albus* were used which, at the time they were inverted in air had lengths of from 2 to 2.5 cm. They remained in air for 36 hours. The roots were then divided into two series, one of which was placed upon the clinostat while the other remained stationary with the root tips directed downward. Of fourteen roots on the clinostat, nine showed a distinct "upward" curvature while five merely flattened the curvature of the tip and then elongated in a straight line. The same number of the fourteen control roots as of the clinostat roots curved "upward." A similar experiment, in which roots of *Pisum sativum* were employed, gave corresponding results. Of twelve of these roots rotated upon the clinostat, seven flattened the original curvature of the tip and bent "upward." Of eleven control roots, similarly treated, save that they remained at rest, four exhibited the upward curvature and all subsequently bent downward into the perpendicular. Other experiments with *Pisum sativum* and *Vicia faba* gave similar results.

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[4] As I shall point out later, the primary geotropic curvature of roots in soil is often suppressed by the resistance of the soil until some time after the reaction of roots in air has begun.

Since the curvatures which Němec considered the result of induced plagiotropism also take place upon the clinostat, they must be considered as autotropic. Not only is there no permanent taking on of plagiotropism by the root in air but the upward curvatures are not even the result of a transitory plagiotropism.

Particularly significant in this connection is the behavior of roots planted horizontally or obliquely upward or inverted in loose moist sawdust behind the glass plate of a Sachs's box. These roots bend downward in a curve of very large radius as is shown by the accompanying figure which is traced directly from photographs.

The radius of curvature in the case of root "B" was 64 cm. For more than forty hours the elongating zone of this root occupied positions from the horizontal to 35° below the horizontal and during this time the extreme tip was directed obliquely downward. In spite of the root tip, as well as the elongating zone, being for so long a time in an extra-perpendicular position, a condition presumably favorable to the induction of the supposed plagiotropic condition, the root, by a uniform curvature, attained a position only 5 degrees from the perpendicular in the subsequent forty-six hours. In the case of another root of the same series of four (see figure 3, *C*) after forty-eight hours growth amounting to 5 cm., the elongating region reached the horizontal. Nevertheless in the subsequent forty-eight hours the elon-

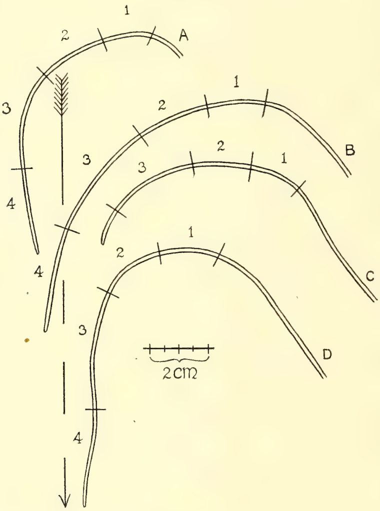

FIG. 3. Tracings from photographs of roots of *Vicia faba* placed obliquely upward in moist loose sawdust in a Sachs's box. The first cross stroke from the right shows the position of the tip at the beginning of the observations. The other strokes show the positions of the tip after the following intervals: 1—24 hours, 2—24 hours, 3—32 hours, 4—24 hours.

gating region reached the perpendicular by a uniform curvature of the root. Roots treated as above but grown at temperatures between 6° and 9° C. often execute so flat a curvature that the horizontal is not reached by the elongating zone for from 8 to 10 days. During this time, in the case of *Vicia faba* and *Lupinus albus* the root may elongate as much as 6 cm. Yet there is no evidence of a plagiotropic condition, for the roots always attain the perpendicular eventually.

Do roots which have undergone geotropic curvature in air lose their geotropic sensibility?

It has already been shown that roots which have grown in an extra-perpendicular position in air for a considerable time curve clear to the perpendicular when brought into earth or even less compact media such as loose moist sawdust. It might be supposed that roots which have performed a geotropic curvature in air lose their sensibility to geotropic stimulus. Sachs, in fact, made this suggestion (1874, pp. 455–456). It is easy to show that such roots are still capable of a geotropic reaction even while growing in air. If a root which has elongated for some time in an oblique direction is displaced until the elongating region is horizontal or inclined upward an active downward curvature takes place, provided only that the root is still actively growing. Of a large number of roots in air which I have tested I have found none in which it was not possible to call forth a distinct geotropic reaction as long as active growth continued.

Do roots diverted from their normal position in earth undergo a reinforcement of their geotropic curvature?

There is no necessity for assuming an agency reinforcing the geotropic curvature of roots in soil in order to explain the oblique position in air and the perpendicular position in soil of roots which have grown twenty to thirty hours in these media after having been placed in a horizontal position. The fact that the root in soil is not free to flatten its primary geotropic curvature is sufficient to explain why the root in soil does not take up an oblique position as does the root in air. It is true that roots growing in air do not generally reach the vertical as the result of the primary geotropic curvature but the incomplete primary curvature of such roots is probably due to interruption of the curvature by the autotropic counter reaction (cf. Simon, 1912, Table X).

But on the other hand the prompt curvature into the perpendicular when placed in soil of roots which have grown in the oblique position in air for several days without showing any secondary curvature does indicate that in the soil some agency is operative which assists the root in its secondary geotropic reaction. Thus if a root which has grown for some time in the oblique position in air is placed in the horizontal it bends downward until the oblique position is again attained. If it is placed horizontally in earth, on the other hand, the secondary reaction does not cease when the oblique position is reached but continues until the elongating zone is in the perpendicular. That this influence of the medium is not dependent upon the material but rather upon its physical properties is indicated by the fact that roots in air and in water behave alike in this respect and those in earth, moist compact sawdust and compressed sphagnum alike curve clear to the perpendicular. In the case of moist sawdust or sphagnum the acuteness of the secondary curvature can be widely varied by compacting the medium to different degrees. When the medium is firmly compressed the curvature of the roots growing in it is as prompt as when soil is employed as a medium. When the sawdust or sphagnum is rendered as loose as possible the downward curvature may be scarcely perceptible even after twenty-four to thirty-six hours. Reference to figure 4 will make clear the difference in the secondary curvature in air and in loose and compact sawdust. The behavior of the root in compact sawdust (represented at *C* in figure 4) differs in no respect from that of roots in earth.

What is the agency which reinforces the curvature of roots in earth and other firm media?

In this section it is my object to consider what reinforcing agency it is which is responsible for the greater acuteness of the secondary curvature of roots growing in earth, sand, compacted sawdust, or compressed sphagnum than in air and in loose sawdust or sphagnum. The media such as moist sawdust or sphagnum the degree of compactness of which can be greatly varied will possess in varying degree, according to the extent to which they are compressed, the following properties which can be conceived of as reinforcing the root curvature: first, moisture content, the amount of water in unit volume of the medium would be increased by compression; second, content of dissolved substance in unit volume of the medium, this would likewise be increased by compression; third, permeability to gases, the rate of gas interchange would become lower as the medium was rendered more compact; and fourth, the resistance offered to the passage of a body through the medium, which would increase as the compactness of the medium was increased. I shall consider in turn the possibility of each of these properties contributing to the difference in the secondary curvature in loose and in compacted sawdust or sphagnum and to the difference in the curvatures taking place in air and in earth. Except when the roots in air are almost constantly sprayed, they are less abundantly supplied with water than when growing in moist soil. Moreover gas interchange is free in the case of roots in air and no appreciable resistance is offered to the advance of the root tip.

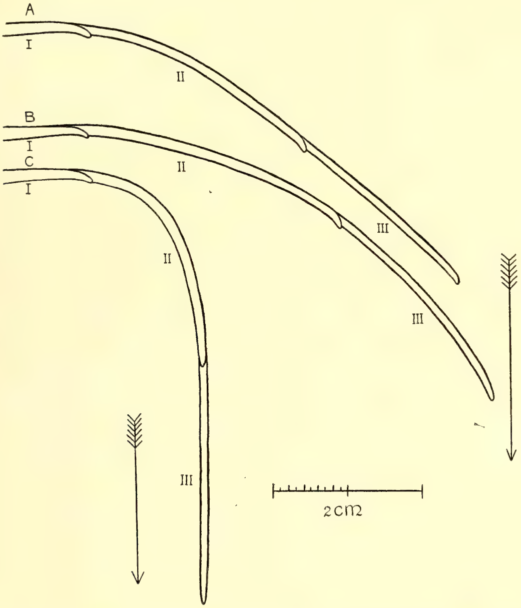

FIG. 4. Curvature of three roots of *Vicia faba* placed horizontally in air (*A*), loose moist sawdust (*B*), and firmly compressed moist sawdust (*C*) after the roots had first curved downward in air and flattened the curvature.

Clearly the amount of water at the disposal of the roots is greater when they are growing in sawdust or sphagnum which is compressed than when they are surrounded by these media in a very loose condition, provided, of course, that, as in my experiments, the proportion, by weight, of water to the medium is in both cases the same. As previously mentioned in connection with the discussion of Hofmeister's theory, any attempt to explain the differences in behavior of the roots in different media as due to differences in water content appears to be finally contradicted by the fact that roots in water behave very much as do those in moist air. It is conceivable however that neither the condition of water shortage prevailing in moist air nor the relatively limited gas interchange in the case of roots submerged in water is favorable to an acute permanent curvature. In this connection I performed experiments with roots which were permitted to curve in response to gravity while growing in earth containing water in different proportions. For this purpose sieved garden earth was employed which was dried in thin layers for four days at 50° C., after which it had lost 28 percent of its weight. Roots planted in this dried soil soon died. The soil was then divided into three lots and water was added to these in the proportions of 20 percent, approximately 30 percent and 40 percent of the dry weight. Six of the eighteen roots of *Vicia faba* var. *equina* used, were planted in each of these three lots of earth in Sachs's boxes. All of the roots curved promptly into the perpendicular. The mean rate of downward curvature was, as is shown by the measurements in Table VI, nearly the same for all three cultures.

In the case of these cultures and those of other similar experiments which I have performed, the curvatures in two lots of earth of which one contained twice as much water as the other (in this case 20 percent and 40 percent of the air dry weight) showed no difference in intensity. In cultures in loose moist sawdust and in moist sawdust so compressed that a given volume contains twice the amount of water in the same volume of the loose sawdust, the curvatures are of entirely different form.

It seems certain that, although differences in the amount of moisture in the medium may not be without influence upon the geotropic curvature, this factor cannot explain the great difference in the secondary curvature of roots in air or very loose sawdust and in earth or compressed sawdust. Furthermore roots in air, even when they are so frequently sprayed that they are constantly covered with a film of water, show no tendency to the acute and complete secondary curvature characteristic of roots in compact non-fluid media.

TABLE VI

Roots of Vicia faba var. equina Performing Geotropic Curvatures in Soils Containing Different Percentages of Water

Percentage of Water to Air Dry Soil	Original Position Horizontal				Original Position Obliquely Upward (150°)			
	Root Number	Angle After 1 Cm. Growth	Angle After 2 Cm. Growth	Minimum Radius of Curvature	Root Number	Angle After 1 Cm. Growth	Angle After 2 Cm. Growth	Minimum Radius of Curvature
20%	1	64°	29°	10 mm.	1	82°	38°	15 mm.
	2	37°	17°	10 "	2	16°	9°	8 "
	3	14°	14°	11 "				
	4	65°	50°	20 "				
28%	1	27°	27°	8 "	1	77°	35°	13 "
	2	52°	29°	17.5 "	2	71°	25°	9 "
	3	51°	30°	20 "				
	4	35°	15°	17.5 "				
40%	1	32°	21°	16 "	1	68°	37°	13 "
	2	36°	23°	18 "	2	89°	23°	10 "
	3	41°	18°	17 "				
	4	36°	20°	10 "				
20%	Mean	45°	27.5°	13 "	Mean	49°	23.5°	11.5 "
28%	Mean	41°	25°	16 "	Mean	74°	30°	11 "
40%	Mean	36°	20.5°	15 "	Mean	77.5°	30°	11.5 "

The flat form of the curvature in air and in loose sawdust or sphagnum and the increasing acuteness of the curvature as the latter medium is compressed might, if our observations extended no further, lead us to suspect the presence of some substance having a specific action upon the root by which the sensibility to the geotropic stimulus or the ability to react could be increased. The fact that media of such widely different nature as sphagnum, moor turf and even horn meal influence the curvature of the root as does moist sawdust speaks conclusively against that possibility. Sawdust which has been repeatedly washed with water is not altered in its effect upon the curvature of the root.

In a less compact mass of such a material as moist sawdust or sphagnum, the gas exchange would be more active than when the medium was compressed. In the more compact mass of the medium there would be a greater tendency for carbon dioxide to accumulate and for oxygen to become depleted. The evidence in the literature in regard to the effect of increase of carbon dioxide and decrease of oxygen upon the geotropic reaction of roots (see Pfeffer, 1906, pp. 140, 143 and 145) would lead us to expect a flatter curvature in a more compact than in a less compact medium, if indeed the accumulation of CO_2 and depletion of O_2 reached a degree sufficient to affect the geotropic reaction at all under these circumstances. In water on account of the relative high solubility of CO_2 as well as on account of the hindrance which the walls of the containing vessel would offer to diffusion the effect of the accumulation of O_2 and accumulation of CO_2 would in all probability be more extensive than in earth. Yet, in water, the root behaves very much as in air.

TABLE VII

Measurements of the Resistance Offered by Soil and Loose Moist Sawdust to Penetration by a Glass Rod Having the Form of a Primary Root of Vicia faba var. equina

Medium	Weight Required to Force Rod 44 Mm. into the Medium	Mean of Preceding Values Plus Weight of Rod and Pan (4 Grams)
Loose moist sawdust 6 days after being placed in a Sachs's box	17 grams	
	17 "	
	15 "	16 + 4 = 20
	17 "	
	16 "	
Freshly prepared, loose, moist sawdust	7 "	
	8 "	7 + 4 = 11
	7 "	
	6 "	
Moist sieved garden soil 6 days after being placed in a Sachs's box	80 "	
	80 "	
	85 "	86 + 4 = 90
	95 "	
	89 "	

With compression, the resistance offered to the advance of the root tip in such media as sawdust or sphagnum would vary greatly and in earth it would be relatively high. In air or water, on the other hand, it would be entirely negligible. By means of a glass rod whose extremity was given the approximate form of the root tip of *Vicia* *faba* var. *equina*, I made measurements of the relative resistance offered to the advance of the root tip through certain of the media which I employed. The root model was of one fourth greater diameter than the root after which it was made. The rod was first forced into the medium for one centimeter of its length. Then weights were placed upon a pan fixed to the upper end of the rod until the rod had penetrated a given distance into the material. The determinations given in Table VII are typical of those obtained in all the experiments.

This very considerable difference in the penetrability of the media may be conceived of as influencing the form of the downward curvature in one of two ways; indirectly by making possible contact stimuli of different intensities or directly by mechanically assisting in the geotropic reaction in some such way as I have already suggested. (See p. 282 of this paper.)

It is possible to explain the differences in the rate of secondary curvature of roots in loose sawdust or sphagnum and in these media when compressed or in soil if we accept Sachs's and Němec's assumption that roots of the species which we and they have employed are positively thigmotropic. The more compact the medium the more resistance it would offer to the change in the form of the root which results from the geotropic reaction. The more resistance the medium offered the more intense would be the thigmotropic stimulus and the more intense the reaction. Thus the downward curvature, the sum of thigmotropic and geotropic reactions, would become more and more acute the greater the compactness of the medium. It would not, however, be so easy to explain in this manner the fact, already referred to, that roots which have been growing obliquely downward in air for several days without appreciable curvature promptly bend downward when placed in soil or other compact media. In this case we are at a loss to account for the initial downward curvature which must occur before the root receives that one-sided contact stimulus which Sachs's theory demands. Moreover the evidence for the thigmotropism of terrestrial roots of the species which Sachs, Němec and I myself have used is indeed meager. The curvatures which Darwin (1880, p. 528 ff.) reported to be negative reactions to contact stimuli were shown by Detlefsen (1882, S. 627) and others to have been in all probability traumatropic curvatures. The positive thigmotropism of terrestrial roots, which here concerns us, was first asserted by Sachs (1874, S. 437, 438). He experimented with roots of *Pisum*, *Phaseolus*, *Vicia faba* and *Zea*. The roots were placed horizontally in air with a pin or piece of wood in contact with one side of the root at a point about ½ mm. behind the extremity of the cap. Positive curvatures were observed in the case of some roots. The concave side of these curvatures were toward the pin or piece of wood and the part of the root which had grown past the object lost the curvature. The curvature became permanent only when the part of the root behind the object had discontinued growth, the permanent curvature being at the point of contact with the "stimulating" object. Newcombe (1902, *b*, S. 243 ff.) by a large number of experiments carried out with great care, has demonstrated that when all possibility of injury to the root by the "stimulating" object is excluded, the roots of the species which Sachs employed exhibit no thigmotropic reaction whatever. He looks upon Sachs's curvatures as traumatic in nature, resulting from direct injury to the tissue and consequent lessened rate of growth at the point of contact.

Němec (1901, *a*, S. 87) reported, as evidence of positive thigmotropism in the case of roots of *Vicia faba*, positive curvatures, resulting from placing on one side of the root tips droplets of plaster of Paris and water. Newcombe repeated these experiments (1904, S. 61, ff.) without obtaining any curvatures whatever. He employed only nine individuals and grants on that account that his negative results were no sufficient contradiction of Němec's assertion. However, as Newcombe has pointed out, it is exceedingly difficult to determine whether such curvatures as Němec reported are thigmotropic, chemotropic, hydrotropic or simply the result of prevention of growth of the tissue to which the dried drop of plaster is attached.

I have not thought it necessary to repeat Newcombe's painstaking experiments but have endeavored to supplement them by determining whether a horizontally placed root which had previously grown for some time in an oblique position in air could be induced by contact "stimulation" to bend clear to the perpendicular in air. I used cylinders of different materials to furnish the contact "stimulus." These were in each experiment of such a diameter that the curvature of the surface corresponding to the secondary curvature of the roots when they were removed from the oblique position in which they had been growing in air and were placed horizontal without contact. Thus when the roots were placed horizontal and in contact with the cylinders in air the curvature of the root kept the under side in contact with the cylinder. The cylinders were of three different sorts: glass tubes both smooth and ground, plaster of Paris cylinders well washed in water, and paraffine cylinders the surfaces of which were roughened by rolling in fine sand. *Vicia sativa* and *Vicia faba* seedlings were employed and proper precautions were taken to prevent hydrotropic reactions, the roots being frequently sprayed and the air around the roots being kept very moist throughout the experiments. The results were entirely negative. The roots in most cases remained in contact with the cylinders until the elongating regions of the roots were inclined from 30° to 60° from the perpendicular. They then left the surface of the cylinders and grew nearly straight ahead.

Altogether it may be said that there exists no conclusive evidence that the species Sachs and Němec employed and those which I have employed are thigmotropic. We are certainly justified in assuming that thigmotropism if, as seems highly improbable, it exists in the case of terrestrial roots, is of sufficient intensity to account for the difference of secondary geotropic curvature in air and earth. Still less can it account for the great difference in the curvature in loose and in compact sawdust or sphagnum.

It remains for us to consider whether it is the direct mechanical influence of the medium upon the reaction of the root which brings about the reinforcement of the geotropic curvature. When a root about 2 cm. long is placed in air at an angle of from 45° to 50° from the normal position, a slight downward curvature takes place which is almost completely flattened. (This is shown at *A* in figure 2.) Thereafter the root elongates with curvature, if any, so slight as to be scarcely perceptible. The extreme tip, however, takes on the curvature already mentioned, which is maintained as the root elongates. This behavior of roots directed obliquely downward in air has been described earlier in this paper. If an obliquely directed root at the stage represented in figure 2, *A*3 is planted in soil without changing its position relative to the perpendicular, it soon bends downward into the normal position. Now, as is clear from a glance at the figure referred to, it would be the upper side of such a root and not the lower side which would experience the greater friction against the soil particles as the curved tip is thrust forward through the earth by the increase in length of the region behind it. If such a root were thigmotropic in the sense in which Němec and Sachs have maintained, the transfer from air, earth or other firm medium would be followed by an upward curvature or, if the thigmotropism of the root were weak, at least in a decrease in the intensity of the downward curvature. This is, however, contrary to the observed facts.

It seems rather to be a passive depression of the downward curved root tip due to the resistance offered to its advance by the medium which reinforces the secondary curvature. The downward bent or asymmetrical root tip which is thrust passively forward from behind by the increase in length of the elongating region cannot follow a straight course in a firm medium because of the non-symmetrical application of the considerable force opposing its advance. This is made clearer by the accompanying diagram (figure 5). The outline

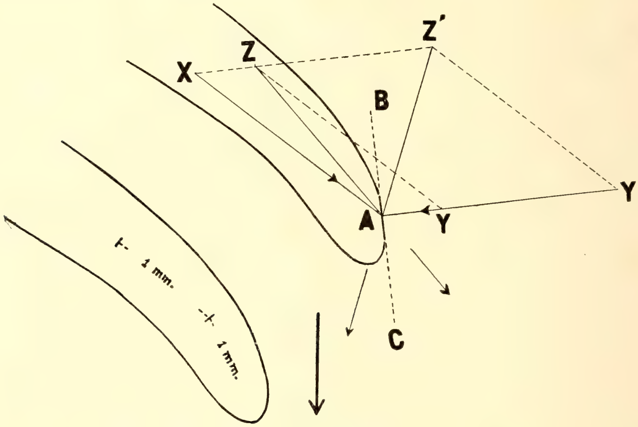

FIG. 5. Diagram illustrating the manner in which the secondary curvature of a root with curved tip is reinforced in a firm medium. The heavy arrow indicates the perpendicular. For further explanation see the text.

below and to the left is from a camera drawing of the tip of a root of *Vicia faba* and in the diagram the same outline has been used except that a portion of the upper surface has been represented as if it were a plane (the solid portion of the line *BC*.) *XA* represents a force acting at *A* and tending to thrust the tip forward in a direction parallel to the axis of the elongating region. This force results from the increase in length of the region of elongation. *YA* and *Y′A* represent respectively the resistances offered by a relatively loose and a relatively compact medium to the advance of the root tip. These forces act at right angles to the surface represented by BC. The resultants of the forces XA, YA and XA, $Y'A$ are respectively ZA and $Z'A$ and their direction indicates a passive depression of the tip. There has been left out of account the friction of the root surface against the particles of the medium, a force which would act parallel to the surface. This force must be small compared with the other forces concerned. It would lessen somewhat the tendency to downward depression of the root tip but probably to only a slight extent.

This effect of the resistant medium in altering the direction of the root's growth by passive displacement of the curved tip is comparable to the effect observed when a stake sharpened to a chisel edge is driven into soil. To drive such a stake straight into hard soil is very difficult and indeed quite impossible if the driving force is applied in a direction parallel to the long axis of the stake. The behavior of the root during its secondary curvature in a firm medium can be illustrated by means of a model root, having three parts; a tip having the form of the curved root tip, an extensible portion representing the region of elongation of the root and a rigid portion, representing the part of the root in which growth has ceased. I constructed such a model in which the extensible portion was formed of small and very elastic rubber tubing. A piece of fine spring wire, which was not very stiff extended through a glass tube which formed the part of the model corresponding to the older part of the root and through the rubber tubing and was attached to the glass "root tip." By means of this wire the "elongating region" could be increased in length and the root tip advanced. When the whole model was "planted" in earth behind the glass wall of a Sachs's box and the tip was pushed forward by means of the wire which extended through a hole in the end wall of the box the "root tip" and the adjacent portion of the "elongating region" were depressed and the "root" bent downward. Just as in the case of the living root, the greater the resistance which the medium offered the sharper was the downward curvature of the root model.

In the case of roots advancing through the soil or other compact media, the resistance of the medium tends to intensify the curvature in still another way. The tip curvature of roots growing obliquely downward, as shown in figure 2 ($A3$, $A4$, and $A5$) is constantly being flattened at the border of the region of active elongation and being reformed at the extremity of the tip. A non-fluid medium hinders this progressive flattening of the tip curvature to a lesser or greater extent according to the compactness of the material. The diagrams (figure 6) of successive stages in the curvature of a root in very loose sawdust,

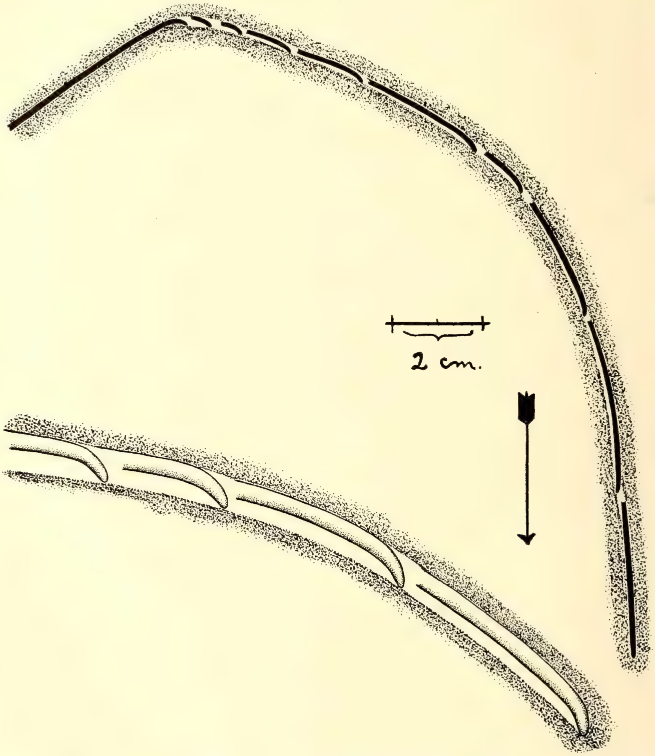

FIG. 6. The tip of a root of *Vicia faba* at different stages in the downward curvature of the root in loose moist sawdust. The upper drawing is from a tracing of the object itself. Below is a diagram representing the same object on a larger scale.

illustrate this point. So little resistance was offered by the medium in the case of the root illustrated in the figure that the sawdust was pushed aside by the curved root tip and thus a channel was formed considerably wider than the root. (The more compact the sawdust the narrower this channel until in very compact sawdust the channel is not wider than the root and there is no free space below the older parts of the root such as is shown in figure 6.) Not only was the root only slightly depressed in the manner described above but also the progressive flattening of the curvature of the tip was almost complete. It is clear that in a medium which would considerably hinder or prevent the flattening of the curvature of the tip of the root the normal position would be reached more promptly and by a more acute curvature than in the case represented in the figure.

The extent of the reinforcement of the geotropic curvature due to the resistance offered by the medium depends upon two factors: the sharpness of the tip curvature and the consistency of the medium. We would expect the rate of curvature to become less as the root approaches the vertical for as the tip approaches the normal perpendicular position its curvature becomes less acute. This is frequently the case when very loose sawdust is the medium used, although the greater compactness of the medium in the lower part of the Sachs's box results in a greater resistance being offered to the advance of the root and frequently makes the difference in the rate of downward curvature inappreciable. It was not found possible to vary the resistance offered by the soil sufficiently to secure such flat secondary curvatures as those in loose moist sawdust or sphagnum except by mixing other materials with the soil. However, roots placed horizontally or inclined upward in fine, moderately moist soil so that the tip was not more than 10 to 15 mm. below the surface often curved very slowly downward during the first 2 to 3 cm. of growth. In these cases the slight weight of soil above caused the soil about the roots to offer little resistance to the advance of the root tip. When a somewhat deeper level was reached, where, owing to the weight of the earth above, a greater resistance was offered to the root's advance, the curvature became more acute and the perpendicular position was soon attained. Sometimes, however, the curvature was so slow that the roots actually emerged from the soil and grew upward into the air.

In this section we have considered a number of points of difference between the media in which the secondary curvature of primary roots is acute and those in which the curvature is relatively flat or lacking, as is often the case in air after the roots have reached an oblique position. It seems to be the last of these points of difference which we have considered, that is, the varying resistance offered by the different media to the advance of the root tip, which is the only one concerned to any extent in bringing about the differences in curvature. This varying resistance does not influence the form of the curvature by making possible a thigmotropic reaction which assists the geotropic curvature. It is rather the direct mechanical effect of the medium, and probably that alone, which causes the differences in secondary geotropic curvature. In the next section experiments will be reported which had for their object the determination of the effect of the medium upon the primary geotropic curvature, that is, upon the curvature which roots which have previously grown in the normal position perform when placed in a position of one-sided geotropic stimulation.

What influence does the medium exert upon the primary geotropic curvature of primary roots?

So striking is the difference in the secondary curvature in media offering different degrees of resistance to the advance of the root tip that the question naturally suggests itself, whether or not the primary curvature also is influenced by the resistance of the medium. The only reference which I have found relating to the course of the primary curvature in different media is the comparison made by Sachs (1874, S. 444–445) of the curvatures of roots of *Vicia faba*, *Pisum*, *Phaseolus* and *Aesculus* placed horizontally in air and earth. He stated that after 4 to 6 hours at 18° to 20° C. the roots growing in both media took on the form of an arc of a circle, the curvature involving the whole growing region. He stated further that after that time the radius of the curvature decreased, the decrease in curvature radius being most active in the region of most active elongation. Thus the curvature took on a parabolic form. After this the first difference in the form of the curvature in earth and air was observed by Sachs. He stated that in the latter medium the regions above and below the most strongly curved zone straightened somewhat so that the curvature became more localized and acute. Roots in earth, on the other hand, although somewhat more acutely bent in the elongating region than the roots in air, also showed considerable curvature above and below this zone. Sachs stated that the roots first showed this difference in the form of their curvatures a little time before the roots in air had reached their maximum curvature.

Now I have attempted to follow somewhat more closely than did Sachs the curvatures in air and in non-fluid media. Although it is my intention to study further the relation of the medium to the course of the primary curvature the results of my experiments have been so uniform that it is appropriate to report them in this connection. Instead of recording the course of the curvature of the roots in written notes and free-hand drawings, I made photographs of the roots at intervals during the primary curvature. The roots which grew in air were mounted on corks which were fixed in crystallizing dishes as already described (see page 278 of this paper) and throughout the course of the curvature the roots remained in the same position relative to gravity, their position not being changed while the photographs were being taken. For comparison with air, moist sawdust was used instead of earth because the penetrability of the sawdust could be readily varied and because, when soil was used as a medium, fine earth particles frequently came to lie between the root tip and the glass wall of the Sachs's box in which the roots were grown. Thus the form of the root was obscured. Repeated experiments had shown that the curvatures in earth and in moderately compressed moist sawdust were not appreciably different. Seedlings of *Vicia faba* var. *equina* and var. *major* were employed and photographs were made at frequent intervals of the course of the curvatures in air, in loose sawdust and in moderately compressed sawdust. While the roots were curving most actively, the intervals between exposures were shorter than when the change in the form of the roots was less active. Thus in the case of one series of photographs of roots growing at a temperature of 23° to 24° C., the intervals between the exposures were 2 hours, 1 hour, $2\frac{1}{2}$ hours, 6 hours, and 12 hours. The source of illumination employed was a 100 cp. Nernst lamp. The light passed through a glass box 10 cm. thick, which was filled with water, before falling upon the roots. The time of exposure varied from $\frac{1}{2}$ to 2 minutes according to the magnification used. The magnification was the same for all the photographs of a given series. The magnifications used in different series varied from 2 × to 4 ×. The curvatures of the roots were compared by superimposing the negatives and viewing them by transmitted light and also by mounting prints from the negatives upon co-ordinate paper. Marks made upon the roots by means of Chinese ink and which appeared in the photographs made it possible to so mount the prints upon the co-ordinate paper that the course of the curvatures could be easily studied and compared. No roots were used which were longer than 6 cm. and the roots under comparison in any series were of the same length.

In the case of roots placed horizontally in air, loose sawdust and compressed sawdust, the first evidence of the beginning of the geotropic curvature was a slight downward asymmetry of the root tip. This appeared at about the same time in the case of the roots in air and of those in loose sawdust and before the roots had undergone any appreciable elongation. Roots in compact sawdust, on the other hand, did not exhibit an asymmetry of the tip until they had undergone an appreciable elongation. (See *S2* and *A2* in figure 7.) The first ap-

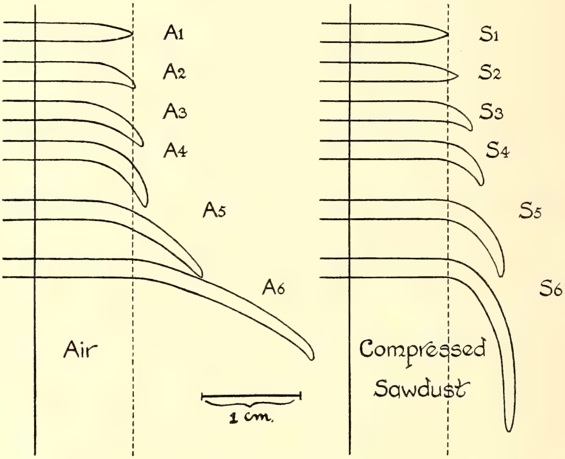

FIG. 7. Diagram showing the course of the primary curvature of main roots of *Vicia faba* placed horizontal in air and in compact moist sawdust. The heavy perpendicular lines cross the roots at points originally 1 cm. from the extremity. The broken lines indicate the original position of the extremity of the roots. For further comment see the text.

pearance of asymmetry in the case of the roots in the compact medium was generally 1 to 2 hours (at 20° C.) after the roots in air had shown the first trace of a reaction.

From the time of the appearance of the first trace of curvature in the case of roots in air the curvature involved more and more the region behind the extreme tip until the zone of most rapid elongation began to curve. (See *A*3 in figure 7.) Meanwhile the roots in compact sawdust had curved downward somewhat. The curvature was restricted, however, almost entirely to that part of the root which was now beyond the original position (indicated in the diagram by the broken lines) of the root tip. In other words the region of the root which lay behind the original position of the tip was curved little if any. (See *S*3.) The difference in the curvature of the roots in air and of those in the compressed sawdust up to this point seemed to have its cause in the relatively great resistance offered by the compact medium to any lateral displacement of the root. The terminal 4 to 5 millimeters of the roots in air were free to undergo a considerable downward displacement. (See *A*3 in figure 7.) This "swinging" displacement of the part of the root beyond the region of most active elongation was in some cases so extensive that a part of the root came to lie 2.5 to 3.5 mm. below the original position of the tip.

After the region of most rapid elongation had become involved in the curvature the bending of the roots in air continued, principally by the activity of the region of most rapid elongation, until the maximum curvature was reached. As the maximum was approached the bending became slower. In most cases the maximum curvature of the roots in air did not amount to 90°. Up to this point the roots in very loose sawdust behaved in a manner similar to those in air, except that the "swinging" downward displacement was hindered considerably by the resistance of the medium. It is clear that the loose sawdust much more effectively opposes the lateral displacement of 3 to 4 mm. of the root than it does the advance of the root tip in the direction of the root's axis. As a result the maximum primary curvature of the roots in moist loose sawdust was considerably less than that of the roots in air. The roots in the compact medium bent promptly and acutely downward as soon as the terminal 2 to 3 millimeters of the roots became distinctly curved. As the curved extremity of such a root was pushed forward through the firm medium by the increase in length of the elongating region, there ensued a sharp curvature downward into the perpendicular, just as in the case of the secondary curvature of roots in such media which has already been discussed. Thus in spite of the fact that in the compact sawdust the beginning of visible reaction was considerable later than in moist air the roots in the former medium frequently reached the perpendicular before the roots in air had attained their maximum curvature. (See *S*4 and *A*4 in figure 7.) The curvature of the root in loose sawdust at the time the roots in air and the compacted sawdust had reached their maximum curvatures was less than either; less than that of the root in air because even the loose sawdust hindered the "swinging" movement resulting from the curvature of the zone of elongation and less than that of the root in compact sawdust because the resistance offered by the loose medium to the curved tip as it was thrust forward by the growth of the root was insufficient to rapidly depress the root tip.

These differences in the primary curvature of roots in the different media used are due apparently to the different degrees of resistance offered by the media to change in the form of the root and to the advance of the tip. On account of the relatively great resistance offered by the compressed sawdust to change in the form of the root, the beginning of the reaction is delayed in the case of roots in this medium. When curvature does begin it is largely restricted, as has been said, to the part of the root which has advanced beyond the original position of the tip. In air the root is quite unhampered in its reaction and the curvature begins sooner and the zone of active elongation takes a more active part in the curvature than in the compact medium. In loose sawdust the curvature is at first very slightly affected by the resistance of the medium. The root is able to displace easily the particles of the loose sawdust and at first the curvature is similar to that of roots in air. The medium lying just below the curving region is compressed by the bending of the root and the resistance offered to further bending increases until it is sufficient to prevent further active curvature of the elongating region. Subsequent curvature is rather favored than hindered by the resistance of the medium, for the extreme tip becomes acutely curved, that is, takes on the tip curvature which has been described earlier in this paper, and, being pushed forward by the elongation of the region behind it the root curves slowly downward in the manner of roots undergoing secondary curvature in the same medium. (See pages 307 and 308 of this paper.)

CONCLUSIONS

1. That the difference in the behavior relative to gravity of roots in air and in earth is not due to differences in the amount of water in the media.

2. That the difference in behavior is not the result of change in the geotonus of the roots, due to their stay in air, whether weakening or loss of geotropism, as Sachs suggested, or assumption of plagiogeotropism as Němec reported.

3. That, as was shown by experiments with media the resistance of which to the root's advance could be widely varied, the failure of the roots in air to reach the vertical is due to the absence of mechanical resistance to the advance of the root tip through that medium.

4. That the secondary curvature of roots in earth, sand, sawdust, sphagnum or other such media is complete because the resistance of these media to the advance of the curved root tip causes passive depression of the root and prevents the complete flattening of the tip curvature.

5. That thigmotropism is not a factor in the difference in the behavior of roots in air and in earth or other non-fluid media.

6. That the resistance offered by the medium to movements of the root tip influences not only the course of the secondary curvature but also the course of the primary curvature, that is, the curvature directly following the placing of the root in a position of stimulation.

The preceding conclusions apply in their entirety to the three principal species employed, *Vicia faba* L. (var. *major* and var. *equina*), *Lupinus albus* L., and *Pisum sativum* L., although a number of forms, mostly Leguminosae, were employed in some of the experiments.

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A STUDY OF DEVELOPMENT IN THE GENUS CORTINARIUS

Gertrude E. Douglas

The genus *Cortinarius* has always been one of particular interest to me on account of the exquisite beauty of its coloration and, in certain species, the peculiar delicacy of its cobwebby veil. It seems strange that a structure as fragile as the latter could be at the same time so persistent, continuing, as it does, until the fruit body has pushed its way well up through the hard particles of soil. It was, therefore, with considerable delight that, while looking for material for morphological study, in company with Professor Atkinson, we ran across *Cortinarius distans* and *Cortinarius cinnamomeus* in all stages of development. This collection, with that of three other species, previously fixed and embedded by Professor Atkinson, furnished the material for the study described in this paper.

The development of *Cortinarius* has never been studied. Kauffman,[1] in his systematic work on the genus, pointed out the need of a more thorough study of the young stages, in order to determine the exact origin of the universal veil and the cortina. Since this work was published, the confusion in regard to the veils in a number of the Agaricaceae has been cleared away by the studies of Atkinson concerning the homologies of the veil in species of *Agaricus*[2,3,4], *Lepiota*,[5] *Amanitopsis*,[6] and *Coprinus*.[8] In the light of this recent work, it becomes important that other species of the family *Agaricaceae* be investigated in regard to the homologies of their veils and the manner of formation of their gills, especially since the exact origin of the latter in the genus *Coprinus*[7,8] has recently become a matter of some controversy. This investigation was accordingly undertaken in order to determine (1) the method of development of the universal and partial veils; and (2) the exact origin of the gills in these five species of *Cortinarius*.

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[1] Kauffman, C. H. The genus Cortinarius, a preliminary study. Bull. Torrey Club **32**: 301–325. 1905.

[2] Atkinson, G. F. The development of *Agaricus campestris*. Bot. Gaz. **42**: 241–264. pls. 7–12. 1906.

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[4] Atkinson, G. F. Morphology and development of *Agaricus rodmani*. Proc. Amer. Phil. Soc. **54**: 309–343. pls. 7–13. 1915.

[5] Atkinson, G. F. The development of *Lepiota clypeolaria*. Ann. Mycol. **12**: 346–356. pls. 13–16. 1914.

[6] Atkinson, G. F. The development of *Amanitopsis vaginata*. Ann. Mycol. **12**: 369–392. pls. 17–19. 1914.

Collection and Preparation of Material.—The material for this investigation was all obtained in the vicinity of Ithaca, N. Y. *Cortinarius distans* Peck and *C. cinnamomeus* Fries were collected in the woods on the south side of Taughannock Gorge, August 27, 1914. The young fruit bodies were dug from rich leaf mold, through which the spawn was running, in the vicinity of mature plants. They were immediately fixed in medium chrome-acetic acid. *C. armillatus* Fries, *C. lilacinus* Peck and *C. anfractus* Fries were collected in a similar manner by Professor Atkinson, the former from the moor at Malloryville, N. Y., and the two latter from the woods by Michigan Hollow swamp near Danby, N. Y., in September, 1914.

The material was dehydrated, cleared in cedar oil and embedded in 52° paraffine. About 300 slides were made of the five species. Sections were cut 5 and 6 microns in thickness. Basic fuchsin proved a most satisfactory stain for *C. cinnamomeus* and *C. distans*, but would not take well in the tissues of the other plants. *C. anfractus* stained well with carbol fuchsin, but *C. lilacinus* proved most resistant, even to this heroic treatment. At the suggestion of Professor Atkinson, a little experimenting was carried on to determine the effectiveness of tannic acid as a mordant, after the sections were fixed to the slide. This substance has generally proved very satisfactory in the case of fungi, when used at the time of killing. It was found that, if the slides were allowed to stand in a 1–2 percent solution for a half-hour and then washed for fifteen minutes in running water, they took most readily the fuchsin and methyl blue stains without precipitation. Sections of *C. lilacinus* and *C. armillatus* were stained in this manner as well as with iron-alum haematoxylin. The fuchsin, however, proved far superior to the others for the photographing.

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[7] Levine, M. The origin and development of the lamellae in *Coprinus micaceus*. Amer. Journ. Bot. 1: 343–356. pls. 39, 40. 1914.

[8] Atkinson, G. F. Origin and development of the lamellae in Coprinus. Bot. Gaz. **61**: 89–130. Diagrams I–VI. pls. 5–12. 1916.

CORTINARIUS ANFRACTUS

(Figs. 1–18)

Primordium of the Basidiocarp.—The earliest stage obtained of *C. anfractus* was a tiny button $\frac{1}{4}$ mm. in length and somewhat conical in shape. But for its attachment to slightly larger fruit bodies, it might easily have been overlooked on account of its diminutiveness (fig. 1). The tissue is homogeneous in character, being composed of densely interwoven hyphae, averaging 1.6 μ in diameter. In certain places on the surface, there is some evidence of a thin, deeply stained external layer, the protoblem. This name was proposed by Atkinson for the very delicate primary universal veil in *Agaricus campestris*[9,10], which separates early from the pileus in the form of very distinct and delicate floccose scales and never becomes concrete with the pileus, as does the true universal veil or blematogen. Owing to the fact that these buttons develop beneath the soil, and because of the friction in washing, it is not strange that only traces of this layer are found in the young stages.

Differentiation of the Pileus and Stem Primordia.—The first internal evidence of differentiation occurs in fruit bodies of about 1 mm. in length. In the central part of the fruit body there appears a deeply staining growth region, conical in form, leaving on the outside an area of loose ground tissue (fig. 1). This conical growth area is the stem primordium. We may consider the outer region as a portion of the blematogen, homologous to that described by Atkinson in the species of *Agaricus*,[11,12] *Lepiota*,[13] *Armillaria*,[14] *Coprinus*,[15]

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[9] Atkinson, G. F. The development of *Agaricus arvensis* and *A. comtulus.* Amer. Journ. Bot. **1**: 3–22. pls. 1, 2. 1914.

[10] Atkinson, G. F. Homology of the universal veil in Agaricus. Mycol. Cent. **5**: 13–20. pls. 1–3. 1914.

[11] Atkinson, G. F. The development of *Agaricus arvensis* and *A. comtulus.* Amer. Journ. Bot. **1**: 3–22. pls. 1, 2. 1914.

[12] Atkinson, G. F. Morphology and development of *Agaricus rodmani.* Proc. Amer. Phil. Soc. **54**: 309–343. pls. 7–13. 1915.

[13] Atkinson, G. F. The development of *Lepiota clypeolaria.* Ann. Mycol. **12**: 346–356. pls. 13–16. 1914.

[14] Atkinson, G. F. The development of *Armillaria mellea.* Mycol. Cent. **4**: 113–120. pls. 1, 2. 1914.

[15] Atkinson, G. F. Origin and development of the lamellae in *Coprinus.* Bot. Gaz. **61**: 89–130. Diagrams I–VI. pls. 5–12. 1916.

Amanitopsis.[16] The loose character of the tissue is due to the less active growth of this region, the hyphae elongating but producing no new elements. As in the case of the species mentioned, the exact limits of this region are impossible to define in the early stages. In the right-hand object of figure 1 this internal growth area has reached the apex of the young fruit body. The upper end of this probably represents the pileus primordium, though there is no evidence of its differentiation from the stem.

It is not until the buttons reach the stage of figure 2 that we can distinguish the separate fundaments of the different regions. At the top of the central growth region, there is differentiated a dome-shaped area, which takes the stain more deeply at its margin. This dome-shaped area represents the fundament of the pileus (fig. 2) and the deeply stained margin, the primordium of the hymenophore. As soon as the latter appears, the fundament of the stem is delineated as that part of the central dense area below the pileus. At the same time the ground tissue between the margin of the pileus and the stem becomes the fundament of the partial veil. From this time on expansion of the fruit body takes place in all directions, particularly in the longitudinal one, and the mesh becomes more and more open (figs. 3 and 4).

The Development of the Hymenophore.—The margin of the pileus fundament (fig. 2) forms an annular zone of very actively growing hyphae, which are becoming very crowded and are turning downward and obliquely outward. They are very rich in protoplasm and contain very prominent nuclei. This annular zone stains deeply and is shown in section in figure 2 as two deeply staining areas one on either side. This annular zone is the fundament of the hymenophore. The hyphae are provided with very sharp ends, thus enabling them to penetrate the ground tissue below the more easily (fig. 10). The hyphae first appear near the stem fundament and as the pileus expands, they are continually being formed at the margin. At the same time, by the branching of the hyphae, new ones are interpolated between the original ones, causing the tissue to become more and more compact.

Origin of the Palisade Layer and Gill Cavity.—When the primordium of the hymenophore is first formed, the growth of the hyphae is very unequal and consequently the lower surface presents the very irregular appearance shown in figures 3 and 10, and in the tangential section of the same fruit body (fig. 4). Soon, however, as new elements are formed in a centrifugal manner, the zone of the hymenophore near the stem begins to change its appearance. The ends of the hyphae become more even and stouter, thus forming the dense palisade tissue shown in figures 5, 6, and 7, and in the slightly older fruit body of figures 8, 9, and 11. It is in this stage that we first find evidence of a gill cavity. The palisade layer becomes very crowded on account of the broadening of the hyphae and the interpolation of new elements. At the same time the growth of the pileus becomes strongly epinastic. These two factors together exert considerable tension on the hyphae of the ground tissue, with the result that they finally break and leave a cavity below the palisade layer. The ragged under surface of the latter in these figures is due to the ends of the broken hyphae. Many seem to be able to stand considerable stretching, so that the cavity at first is rather weak. It is, however, important to notice that before there is any evidence of gill formation, there is a well defined, even, palisade layer.

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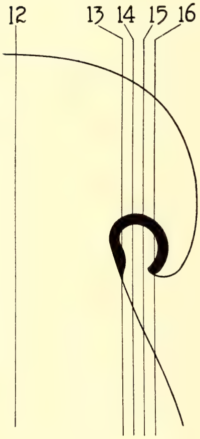
[16] Atkinson, G. F. The development of *Amanitopsis vaginata.* Ann. Mycol. **12**: 369–392. pls. 17–19. 1914.

Origin of the Partial Veil.—We have seen that very early in the life history of the fruit body, the pileus and stem primordia are differentiated by the appearance of a dome-shaped area, growing very actively at its margin and here forming the primordium of the hymenophore. The general region of looser plectenchyma, extending over the pileus, outside the hymenophore and down over the stem primordium, is the blematogen. The ground tissue within this somewhat indefinite zone, lying between the margin of the pileus and the stem fundaments, represents, as before noted, the partial veil. As the plant matures, the upper part of the pileus fundament grows into and becomes consolidated with the blematogen, which forms here a firm cortex. At the sides, however, the blematogen shows a more or less duplex character (figs. 3 and 5). There is a very thin but firm outer layer, which stains very deeply and is composed of two or three rows of parallel or slightly interwoven hyphae with large diameters. Within, the blematogen is very loose and delicate and is exactly similar to the tissue of the partial veil. This double character may be distinguished even on the upper surface of the pileus margin (figs. 3, 5, and 8), where the outer layer merges into the cortex of the fruit body, quite similar in its composition. This rind is very persistent, retaining its integrity until after the gills are fairly developed (fig. 17). It is quite conspicuous even to the naked eye. As the plant matures, new elements appear to be formed at the margin of the pileus. These extend across, below the gill cavity, to the stem. Whether or not the cortina has its insertion on the upper surface of the pileus margin, depends, therefore, on our conception of what the cortina is. *C. anfractus* is placed by systematists in the sub-genus, *Phlegmacium,* one of whose characters is the presence of a partial veil only. We have seen from this study, however, that *C. anfractus* does possess a blematogen outer layer, which is homologous with a universal veil, but is not clearly differentiated from the pileus as such. The marginal or partial veil, strictly speaking, consists only of the ground tissue, between the stem and pileus margin and the new elements added by growth. As the term cortina is a special one, applied chiefly to the cobwebby veil of this genus, it should probably include all the fibers of the veil and thus may be considered as consisting of both blematogen and partial veil tissue.



Text-fig. 1. Diagram to show plane of sections shown in figures 12–16, plate IX.

Origin of the Lamellae.—The first evidence of gill formation occurs in specimens of about 3.5 mm. diameter. Figures 12–16 show a series of longitudinal sections, cut parallel with the axis of the stem, in the positions represented by the corresponding numbers in the diagram of text-figure 1. Let us first examine section 14, from a plane through the center of the gill cavity, recalling, as we do so, that the hymenophore always develops centrifugally and in consequence, the oldest stages will be nearer the stem and the youngest nearer the margin of the pileus. The latter portion of the hymenophore is still in the primordial state (fig. 16) showing the irregular, sharply pointed hyphae. As we approach the stem, we pass by even palisade tissue to the young gill salients (fig. 15) growing down into a now well defined annular cavity. If we examine them in greater detail (fig. 18), we see that they are formed by the dense crowding of the palisade layer, accompanied by an elongation of the subadjacent hyphae in regularly spaced radial areas. The palisade layer is thus pushed out into folds, which allows room for the expansion of the ends of the hyphae, now considerably swollen. Subadjacent to the stratum of young salients, there appears a thin growth zone, very conspicuous on account of its abundant nuclei. In the center of each gill, it extends down into the trama, but between the gills it remains as a thin layer[17] (figs. 13–15, 18). It marks the boundary of the pileus and the hymenophore and probably represents the region where cleavage takes place in such forms as *Paxillus*, in which the hymenophore separates readily from the flesh of the pileus. As we approach the stem, we find the salients more mature. The median section of figure 12 shows that the hymenophore, when cut radially, is more or less crescent shaped, due to the inrolling of the edge of the pileus and the decurrence of the gills along the slanting surface of the stem. As the knife passed through the plane represented by line 13, it cut through the junction of the decurrent gills with the stem and nearly perpendicular to their direction of growth. For this reason, the space between them, a part of the main gill cavity, appears as a little pocket. In a similar manner, the peculiar appearance of figure 16 may be explained. The curving inward of the margin of the pileus caused the hymenophore primordium to be cut twice.

Structure of the Pileus and Stem.—The pileus and stem are both composed of a very homogeneous and yet firm tissue, containing very large air spaces. Toward the outside of the pileus, the tissue becomes more and more dense until it passes into the firm cortex, spoken of above. On the outside of the cortex, we frequently find large, thick-walled hyphae undergoing disintegration and giving the pileus surface the viscid character common to this sub-genus. In the cortical region of the pileus, the hyphae are filled with large oily drops, remaining unstained. At the apex of the stem the tissue is very dense, but it becomes more and more open towards the base, thus providing for aeration of the tissues. In this region also the hyphae are stouter and have thicker walls than those above near the pileus. The general direction of the hyphae in the stem is longitudinal or slightly oblique. They are somewhat interwoven. On the outside of the stem, they are slender and dense, forming a very firm outer supporting layer.

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[17] This recalls the distinct zone from which the hymenophore originates in *Polyporus fumosus* as described by Miss Ames (p. 225, figs. 38, 39). See Ames, A. A consideration of structure in relation to genera of the Polyporaceae. Ann. Mycol. 11: 211–253. pls. 10–13. 1913.

CORTINARIUS CINNAMOMEUS

(Figs. 25–50)

Early Stages of Development.—*Cortinarius cinnamomeus* develops in a manner very similar to that of *C. anfractus*, although there is considerable difference in the appearance of their tissues, that of this species being more dense in its character than that of the preceding. The earliest stage examined is represented by figure 25, from a section of a button about 2 mm. × 1 mm. in size. This has already differentiated sufficiently to show a dense dome-shaped fundament of the pileus and that of the stem below and it is quite possible that earlier stages would show the stem fundament differentiated first as a conical area, as described for *C. anfractus* and for the other species described in this paper. The hyphae of the pileus fundament average about 1.6 μ in diameter but in the lower part of the stem and in the veil region they reach two or three times this size. The pileus primordium lies very near the surface, leaving only a very narrow thin zone of blematogen. The latter is composed of large hyphae with diameters of 4 or 5 μ. They are somewhat interwoven and are arranged more or less radially over the surface of the pileus, excepting in the hymenophore region, where they are very nearly parallel to the surface. This blematogen layer is retained by the plant until maturity and accounts for the fibrillose nature of the surface of the pileus, a distinguishing characteristic of the sub-genus *Dermocybe*, to which *C. cinnamomeus* belongs. It never separates as a universal veil but is partly worn off in the form of scales, as the fruit body pushes through the soil (figs. 32, 39, 44). The lost fibrils appear to be continually replaced by outgrowths from the surface of the pileus. The marginal veil never becomes very strongly developed. It receives new elements from the margin of the pileus (figs. 28, 30), but it retains its loose floccose nature. The large open mesh provides a very free communication for air with the outside.

The Development of the Hymenophore.—Simultaneous with or soon after the formation of the pileus fundament takes place, an internal annular zone of rapid growth appears at its margin and represents the beginning of the hymenophore. This primordial stage is followed by the development of the palisade layer (figs. 27, 28, 29, 30, 31). Owing to the weakness of the fibers of the ground tissue, a strong gill cavity makes its appearance early (figs. 29, 30). In figures 32–38 is shown a series of sections of the earliest fruit body to show the gill beginnings. Excepting for slightly more irregularity of the salients, their development is quite similar to that of *C. anfractus*. In this specimen the gills are merely adnate to the stem and, in consequence, do not show the pockets at the junction of the stem, which we saw in *C. anfractus*. There is considerable variation in this respect among individuals of the same species. The older specimen, shown in figures 39–43, has decurrent gills and in consequence we find the pockets present. This series of sections is interesting on account of the beginning of the secondary gills. The primary ones have already reached a considerable degree of development, when the second series begin to appear (figs. 45–49). As the pileus expands, the primary salients are pushed farther and farther apart by the intercalary growth and finally the new salients begin to form. At first the surface between the original gills is even. Then the ends of the hyphae become swollen and there is considerable crowding, especially next the primary gills (fig. 45). This is followed by an elongation of the hyphae, carrying the gill down into the cavity. The hyphae in the central portion grow straight downward, but their dense crowding and swollen ends cause the others to turn outward. In the course of development, many new hyphae are interpolated between the old, contributing also to the increase in length. The terminal cells of the hyphae take the stain with difficulty, showing that the greater activity in growth is behind the tips, where the stain is deep and the nuclei abundant. We find in this species the same narrow deeply staining growth region subadjacent to the salients and extending down into their trama (figs. 40–43, 48, 49), that we found in *C. anfractus*. Figure 43 may need a word of explanation. The section was cut somewhat obliquely, so that it passes through the gill cavity on one side, but through the margin of the pileus on the other. It thus passes perpendicularly to the direction of growth of the gill salients on one side, thereby forming pockets as on the stem. This results from the strongly incurved margin of the pileus (see figs. 40–42). In figure 50 is represented a section of the hymenophore from a fruit body having mature gills.

CORTINARIUS ARMILLATUS

(Figs. 19–24)

Cortinarius armillatus and *Cortinarius distans* are of especial interest on account of the fact that both belong to the only subgenus, *Telamonia*, described as possessing a universal veil. *C. armillatus* derives its name from the fact that, as the veil breaks away from the margin of the pileus, it is left on the stem in a series of rings. The material was not found in great abundance but represents the most critical stages of development, except the origin of the hymenophore. The youngest stage (fig. 19) already shows a differentiation into three regions. In the center there is a region of active growth, conical in shape, which probably represents the stem fundament. This is surrounded by a zone of ground tissue, on the surface of which is a layer of large thick-walled hyphae. The latter is probably not a protoblem, evidence of which shows in the older fruit bodies, but is perhaps due to changes in the hyphae, caused by some substance in the substratum with which it came in contact. The older specimen, represented in figure 20, shows a considerable increase in the central growth zone, which now extends upwards nearly to the apex of the young basidiocarp. Progression of the growth area of the stem fundament upward gives rise to the pileus fundament which is surrounded laterally by loose ground tissue and blematogen, there being evidence of a slight but broad constriction between pileus and stem fundament. This method of differentiation of stem and pileus fundament is like that described by A. Möller[18] (p. 70) for *Rozites gongylophora*, and by Atkinson[19] for *Lepiota cristata* and *seminuda*.

On the outside is a very delicate layer of fibrils, which in all probability here represents a true protoblem. It is present in all the older stages where we find very delicate fibrils or scales gradually being shed by the plant. For this reason, we may assume that it is also present in the early stages, but was lost from the button of figure 19 during the preparation processes. The zone just within represents the blematogen, a region very indefinite in the young stages. As the plant becomes older, the boundaries of the blematogen become more distinct

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[18] Möller, A. Die Pilzgärten einiger südamerikanischer Ameisen. Bot. Mittheil. Trop. **6**: 1–127, figs. 1–4. pls. 1–7. 1893.

[19] Atkinson, Geo. F. The development of *Lepiota cristata* and *L. seminuda*. Proc. 20th Anniversary N. Y. Bot. Gard.

(figs. 21 and 22) until, in the mature stages, it becomes a very definite area. Its duplex character shows even more distinctly here than in *C. anfractus*. The outer layer is continuous completely over the surface of the pileus, the gill cavity and the upper part of the stem. It is considerably firmer than the floccose portion within, which extends from the upper surface of the pileus margin to the stem, outside the partial veil. The latter is very fragile and as the epinastic growth of the pileus takes place, the hyphae are considerably stretched and become united in strands (fig. 23). The partial veil gradually breaks loose from the pileus margin and, together with the portion of the blematogen outside with which it remains in contact, is torn apart by the elongating stem so that the series of delicate rings, above described, are left around the stem. The stages of early development of the gills are lacking but figures 21 and 22 show that they are preceded by a palisade layer and extensive gill cavity. Figure 24 represents a later stage of the gills and shows the origin of a forked gill, by means of a secondary salient growing out at the base of a primary gill.

CORTINARIUS DISTANS

(Figs. 63–69)

A few photographs of *Cortinarius distans*, also belonging to the sub-genus *Telamonia*, are shown in figures 63–69. These were chosen to illustrate the character of the veil and the method of development of the gills. A very distinct blematogen layer covers the fruit body. Its hyphae are characteristically large and generally radial in their arrangement over the apex of the fruit body, but become nearly parallel at the sides. They take the stain very lightly. The blematogen soon breaks into scales and becomes easily removed, exposing the surface of the pileus, made up of very firm pseudoparenchymatous tissue (fig. 69). The pileus retains its conical shape as it pushes through the soil and on this account, the blematogen first disappears from the apex and much later at the margin of the pileus. Its floccose character is shown in figure 64, an enlargement of figure 63. On account of the scaling off of the outer layers, it was not determined whether or not the blematogen was originally duplex.

The method of gill formation is like that of *C. anfractus*, with the exception that the salients are broader and more widely distant, resulting in a triangular gill at maturity. On account of this feature, the plant has received its name of *C. distans*.

CORTINARIUS LILACINUS

(Figs. 51–62)

The sub-genus *Inoloma,* to which *C. lilacinus* belongs, is characterized by the presence of a large bulbous base to the stem, a feature which in some species becomes extremely well developed. Before there is any external sign of differentiation, a good-sized tubercle is formed (4 × 5 mm.), within which certain changes are taking place. Figure 51 represents a median section of such a tubercle. The tissue is very nearly homogeneous in character, with the exception of a somewhat more dense, deeply staining region within, broadly conical in form, the primordium of the stem. The next later stage (figs. 52, 53) exhibits a considerable advancement in development. The whole of the lower part of the tubercle has become very dense and compact. Above, the hemispherical pileus primordium is marked off from that of the stem by the deeply staining hymenophore fundament. The fibers in the pileus region are similar to those in the stem, are very closely massed together and assume a generally radial arrangement. At the margin, however, they turn strongly downward and here form the hymenophore primordium (figs. 52, 61). Extending over the surface of the pileus and to the sides of the tubercle, is the blematogen, a still rather indefinite region (fig. 52). The gills are formed in the manner described for the other species. An annular cavity appears at about the time of, or just prior to, the formation of the palisade layer (figs. 56, 57), which is very quickly followed by the formation of the young gills. The palisade cells take the stain less easily than the subadjacent tissue and thus the subadjacent region stands out in sharp contrast. As in *C. anfractus,* the latter represents a new area of active growth. It remains as a thin stratum between the gills but when it reaches them, it curves downward and growing very actively forms the greater part of the trama.

On the surface of the fruit body, no definite cortex is formed. Although the pileus and the blematogen becomes consolidated, in the older stages the boundary between the two regions becomes very distinct, owing to the difference in the character of the tissues (fig. 62). The outer layers appear to rub off, as the plant pushes up through the soil, so that a duplex character to the veil is here not apparent. The cortina is made up of the blematogen and the partial veil, to which new elements are added by marginal pileus growth. Practically no elongation takes place in the stem until all the parts are well organized. In the mature stage shown in figure 62, the fruit body is just beginning to push up from the tubercle.

SUMMARY

1. In the general features of development, these five species of *Cortinarius* are alike. The first differentiation to take place is that of a rapidly growing more or less conical region, the stem primordium, within the fundamental plectenchyma. Growth and progressive differentiation from the apex of the stem fundament gives rise to the pileus primordium. Quickly following the appearance of the pileus fundament there is developed the fundament of the hymenophore, an internal, annular active growth area at the margin of the pileus primordium. Growth is centrifugal. The primordial zone is changed into that of the palisade and this is transformed into the zone of young gill salients. Before the latter make their appearance, a gill cavity of considerable size is formed, by the tensions of the rapidly growing tissues of this region. The tendency to epinastic growth of the pileus margin and the dense crowding of the interpolating elements cause the weak fibers of the ground tissue below to become stretched and finally broken, leaving the cavity.

2. The gills are formed in the following manner: The cells of the palisade layer increase in diameter. This excess of growth is at the same time taken care of by the growth of the subadjacent cells of the hyphae, which elongate in radial rows at regularly spaced intervals. The gill increases in size by the rapid elongation and increase in number of the tramal hyphae, together with an increase in the elements of the palisade and subadjacent layer.

3. In all five species, whether developing a universal veil or not, there is present a blematogen layer. This becomes evident as soon as the fundament of the pileus and hymenophore appear. Its boundaries at first are very indefinite but in general it represents a zone outside the pileus, marginal veil and stem fundaments. Its later disposition varies in the five species. In *C. armillatus* and *C. distans* it enters into the formation of a "universal veil," which separates from the surface of the pileus, but it does not form a true volva, or *teleoblem*. In the other species, it becomes consolidated with the surface of the pileus; in *C. cinnamomeus* breaking up into fibrils which clothe the surface. A very interesting feature of the blematogen is

its duplex character over the margin of the pileus and partial veil. In *C. anfractus* and *C. armillatus* there is developed a firm outer layer, which persists until maturity. This zone is very thin, but extends completely over the upper part of the fruit body. In *C. anfractus* it becomes consolidated with the cortex. The inner portion of the blematogen is much more floccose in character and extends from the upper surface of the margin of the pileus, outside the partial veil, to the stem. In the other three species, *C. cinnamomeus*, *C. distans*, and *C. lilacinus*, the duplex character is not evident, owing perhaps to the early wearing away of the surface. The inner floccose zone is, however, present in all.

4. The marginal veil is developed from the ground tissue lying between the fundaments of the hymenophore and stem, to which are added new elements from the margin of the pileus. The cortina is therefore made up of two elements; 1st, the blematogen on the outside, which extends from the upper surface of the margin of the pileus to the stem; and 2d, the inner threads consisting of the fibers of the marginal veil.

5. One species, *C. armillatus* seems to possess a protoblem. There is evidence of this layer being present in early stages of another species, *C. anfractus*. It is very possible with other methods of fixing and preparation, it might be found in all.

In conclusion, I wish to acknowledge my deep indebtedness to Professor G. F. Atkinson for his kind direction and helpful advice.

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DESCRIPTION OF PLATES VIII–XIII

Figs. 10, 11, 18, 31, 44–49, and 61 were taken with a Bausch and Lomb compound microscope, fitted with a Zeiss 4 mm. ocular and 4 mm. objective. In fig. 64 an 18 mm. oc. and 16 mm. ob. were used. The other figures were photographed by means of an extension camera and Zeiss lenses, figs. 12, 13, 14, 15, 16, 17, 23, 39, 40, 41, 42, 43, with a 35 mm., figs. 52, 53, 54, 55, 58, 59, 60 with a 50 mm. and the others with a 16 mm. Spencer Lens Co. photographic objective.

Cortinarius anfractus (figs. 1–18)

FIG. 1. × 34 diameters. Early stages of three fruit bodies. The one on the left is still undifferentiated, excepting for a darker layer on the outside, which is possibly protoblem. The two older fruit bodies show an area of active growth within, organizing stem and pileus primordia.

FIG. 2. × 34 diam. A slightly older stage. Median section showing the primordia of pileus, hymenophore and stem.

FIG. 3. × 34 diam. Median section of an older stage. The primordial stage is changing into the palisade layer.

FIG. 4. × 34 diam. Tangential section of the fruit body of fig. 3.

FIG. 5. × 34 diam. Median section of a fruit body with the palisade layer well developed. The outer and inner regions of blematogen are shown and the gill cavity is just beginning to form. On the top of the basidiocarp the blematogen has become consolidated with the surface of the pileus and forms a firm cortex.

FIG. 6. × 34 diam. Tangential section of same, near the stem.

FIG. 7. × 34 diam. Tangential section of same, beyond the stem.

FIG. 8. × 34 diam. Older stage, the last to show the palisade layer before the formation of gills.

FIG. 9. × 34 diam. Tangential section of same.

FIG. 10. × 250 diam. The hymenial region of fig. 3, showing the hymenophore primordium on a larger scale.

FIG. 11. × 250 diam. An enlargement of Fig. 8, showing the well-developed palisade layer.

FIGS. 12–16. × 14 diam. A series of sections, showing the various stages in the development of the gills. Fig. 12 represents the median section. The others are all cut parallel to it from the same fruit body.

FIG. 17. × 14 diam. A section of a nearly mature fruit body, showing the persistent blematogen.

FIG. 18. × 250 diam. An enlargement of the young salients of fig. 15 to show the narrow growth zone, subadjacent to the salients. The very abundant nuclei show prominently.

Cortinarius armillatus (figs. 19–24)

FIG. 19. × 34 diam. Youngest stage obtained. The button has begun to differentiate into a central growth region, probably the stem fundament, and an indefinite blematogen. The outside darker layer probably represents modified blematogen.

FIG. 20. × 34 diam. Slightly older stage showing considerable increase in the central growth region. The layer of loose tissue on the outside probably represents a protoblem.

FIGS. 21 AND 22. × 34 diam. Median and tangential sections of a fruit body in which the palisade layer is developed.

FIG. 23. × 14 diam. Median section from a nearly mature specimen, showing the duplex character of the blematogen and the strands of the marginal veil being torn by the tension in growth of the hymenophore and pileus margin.

FIG. 24. × 34 diam. A section showing mature primary gills, and forking caused by the development of secondary salients at their bases.

Cortinarius cinnamomeus (figs. 25–50)

FIG. 25. × 34 diam. Median section of earliest stage obtained. The pileus fundament is evident as a deeply stained saucer-like area near the top of the button. The blematogen consists of the loose tissue upon the surface.

FIG. 26. × 34 diam. A slightly older fruit body, showing the beginning of the hymenophore primordium.

FIGS. 27 AND 28. × 34 diam. Median and tangential sections of an older stage. The palisade layer is in the process of formation.

FIGS. 29 AND 30. × 34 diam. An older fruit body, showing the gill cavity.

FIG. 31. × 250 diam. An enlargement of the palisade layer and gill cavity of Fig. 29.

FIGS. 32–38. × 34 diam. A series of sections at various intervals between the central axis of the stem and the margin of the pileus, showing the origin of the gills. Note the fibrillose character of the pileus surface in fig. 32.

FIGS. 39–43. × 16 diam. Stages in the formation of the gills of an older specimen. The secondary gills are beginning to form.

FIG. 44. × 250 diam. An enlargement of the margin of fig. 39, showing the pseudoparenchymatous cortex and fibrillose surface of the pileus.

FIGS. 45–49. × 250 diam. Stages in the development of a secondary gill. Fig. 45 is nearest the margin. As the sections approach the center, they show progressive stages of maturity.

FIG. 50. × 34 diam. Nearly mature primary and secondary gills.

Cortinarius lilacinus (figs. 51–62)

FIG. 51. × 34 diam. Median sections through a tubercle, showing the differentiation of the stem fundament in the center.

FIGS. 52 AND 53. × 11 diam. Median and tangential sections of an older specimen, showing the pileus, hymenophore and stem primordia.

FIGS. 54 AND 55. × 8 and 11 diam. resp. Older specimen, showing the organization of the palisade layer.

FIGS. 56 AND 57. × 34 diam. The palisade layer and gill cavity, just before the development of the gills.

FIGS. 58, 59 AND 60. × 11 diam. Sections of the youngest fruit body to show gill formation.

FIG. 61. × 250 diam. An enlargement of the hymenophore region of fig. 52, still in the primordial stage.

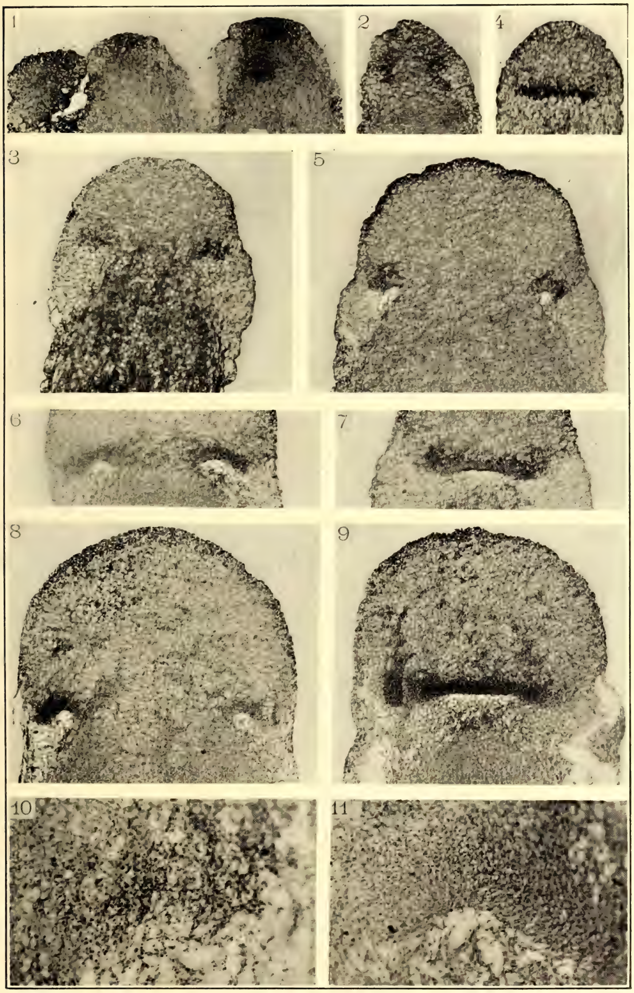
FIG. 62. × 9 diam. A nearly mature fruit body, just commencing to elongate from the tubercle.

Cortinarius distans (figs. 63–69)

FIGS. 63, 65, 66, 67, 68. × 34 diam. A series of sections showing the development of the gills and the flocculent blematogen, which is rapidly disappearing from the surface.

FIG. 64. × 150 diam. An enlargement of fig. 63, to show the character of the marginal veil, the blematogen and palisade regions.

FIG. 69. × 34 diam. Median section of nearly mature fruit body.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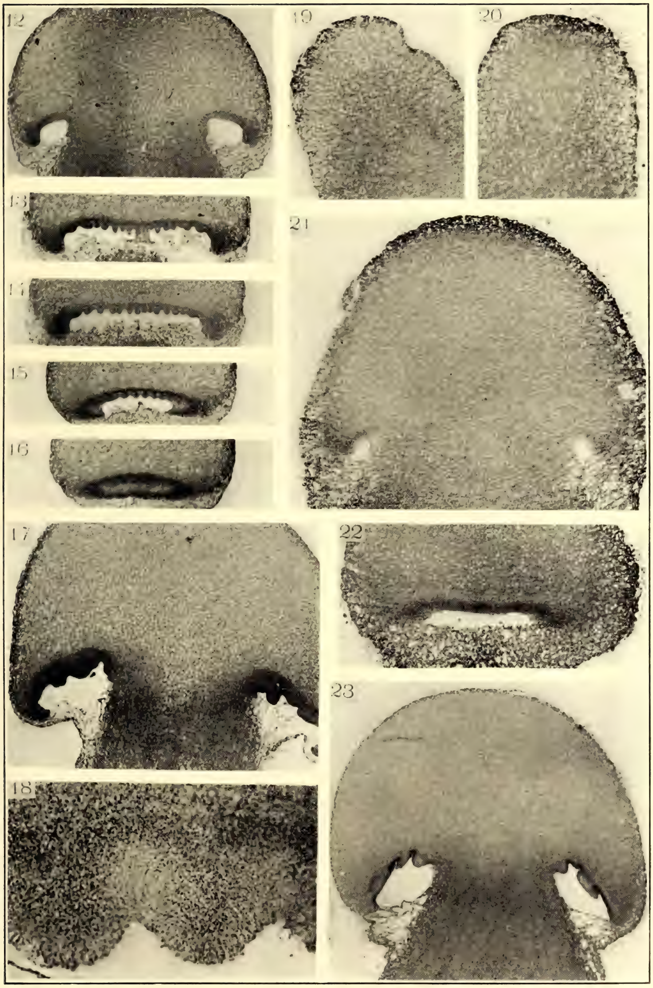
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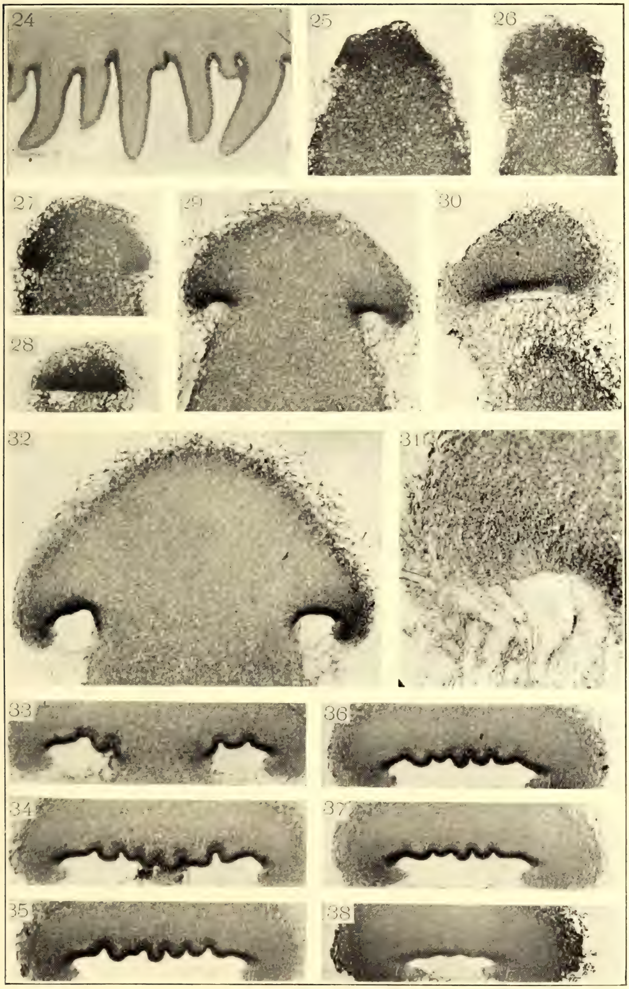
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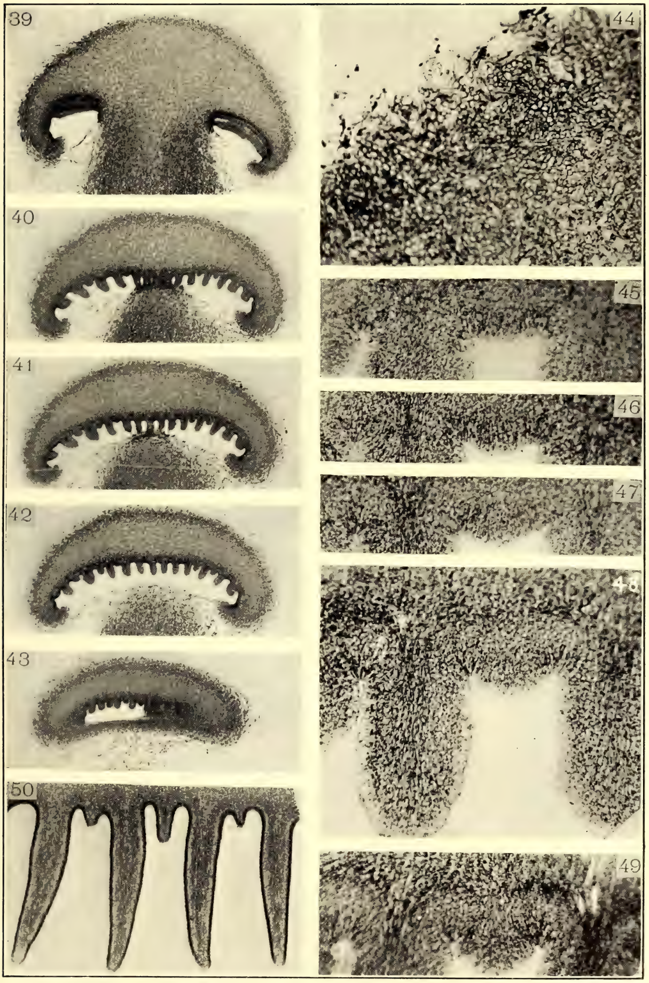
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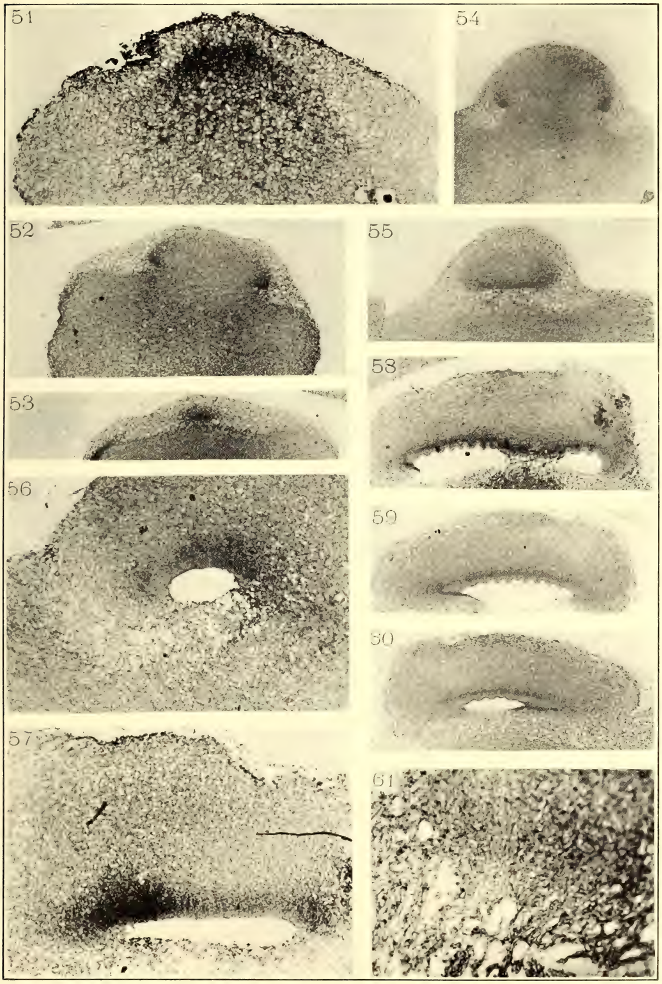
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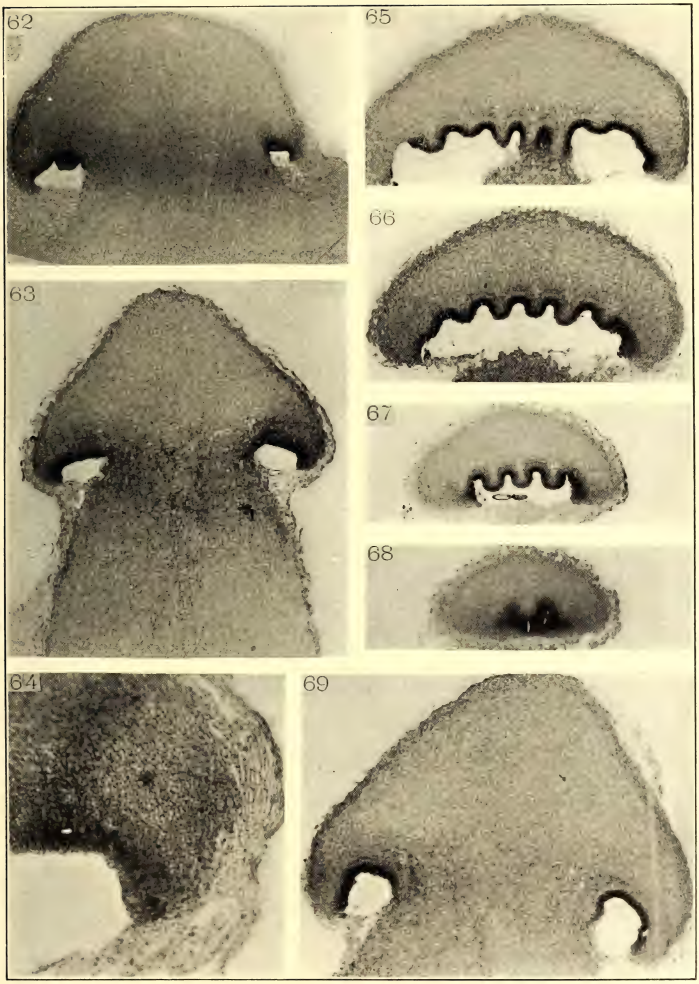
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Douglas: Cortinarius lilacinus (62); C. distans (63–69).

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THE DEVELOPMENT OF THE PHYLLOXERA VASTATRIX LEAF GALL

HARRY R. ROSEN

I. INTRODUCTION

Phylloxera vastatrix Planchon [Stebbins (27) uses the synonym *Phylloxera vitifoliae* Fitch] is the plant louse which was introduced into Europe from America and became one of the most dreaded enemies of the European grape vine. On American vines the insect usually attacks the leaf producing ugly cecidial growths but not seriously impairing the health of the plants attacked. On European vines, however, the insect does most of its work on the roots, becoming a very destructive pest. Much has been written concerning the life history of this parasite, a large part of which has been brought together by Viala (29).

II. OCCURRENCE AND APPEARANCE OF THE MATURE GALL

The material for this paper was gathered in the vicinity of Madison, Wis. The profuse growth of the wild vines of *Vitis vulpina* L., together with the ease with which one can find the Phylloxera galls, made this gall a desirable one to study in this region. My observations extended over a period of two growing seasons, during which time I have been able to observe hundreds of these cecidia. Although Cook (6 and 7) and Stebbins (27) found the galls on both the upper and lower surfaces of the leaves, I have found them only on the lower surface. Houard (15) and Cornu (8) mention only the lower surface as the bearer for the

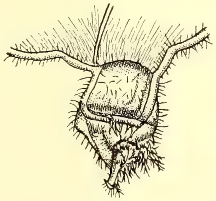

FIG. 1. A gall as seen on the lower surface of the leaf through a hand lens. Magnified about 3 times.

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European galls. Very frequently the lower surface would be completely spotted with these wart-like outgrowths. A good-sized gall is about as large as a pea. It appears, on close examination, as a much furrowed and wrinkled, irregular pouch, with hairy projections, the mouth of the pouch opening on the upper surface of the leaf. When the gall has reached maturity the mouth is marked by two lip-like growths extending 2 to 3 mm. above the upper surface of the leaf, while around these lips a profuse growth of glistening, downy hairs covers and entirely closes the opening to the cecidial cavity.

III. Histology of the Normal Leaf

It is very evident that, in order to understand fully the changes which occur in the *Vitis vulpina* leaf during gall formation, it is necessary to have a good understanding of the structure of the young and of the mature normal leaf. Cook (4) gives a drawing of a normal leaf of *Vitis vulpina* and notes that, as compared with other leaves, the palisade is not pronounced, while the mesophyll is more compact. Comparing gall structure with the structure of the normal leaf, he points out that it is necessary to compare the gall structure with the structure of the leaf on which the gall is found, and not with the typical leaf. I have found, contrary to Cook, a well-developed palisade, and a spongy mesophyll with numerous air spaces in mature, gall-bearing leaves. Text-figure 2 shows a drawing of a cross section of a mature leaf taken from a portion immediately adjoining a fully

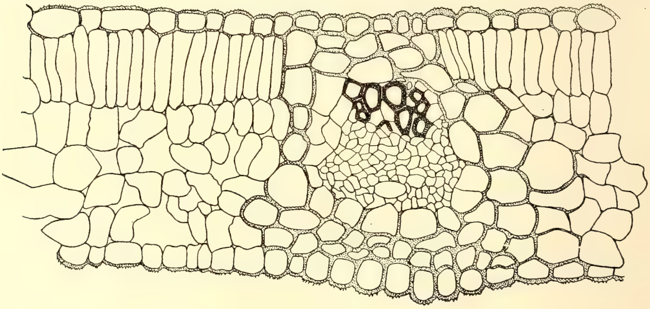

Fig. 2. A cross section of a normal leaf, magnified about 349 times.

developed gall. Cook's figure leads me to suspect that he did not have a fully developed, normal leaf. The figure which he gives of the gall would seem to substantiate this view, since this figure does not appear to represent a typical fully developed gall as Cornu (8) or as I have found it. However, since gall formation always begins on the very young, embryonic bud leaf, it becomes necessary first to consider the structure of this leaf. Text-figure 3 shows a drawing of a cross section of such a young leaf. The diameter of this leaf is about one-half that of the fully developed one; the average diameter of the mature leaf is about 144 μ and of the young leaf, about 68 μ. A close examination of the young leaf figured below shows two epidermal layers with as yet no development of cuticle, an abundance of rather rigid, unicellular hairs around the margin of the leaf and on the larger veins, with a few very sparsely scattered hairs over the lower and upper leaf surfaces, an embryonic palisade layer made up of cells not much longer than they are wide and showing very little variation in length as compared with their width, and an embryonic spongy mesophyll made up of 3 layers of cells, so close together that scarcely any air spaces can be observed.

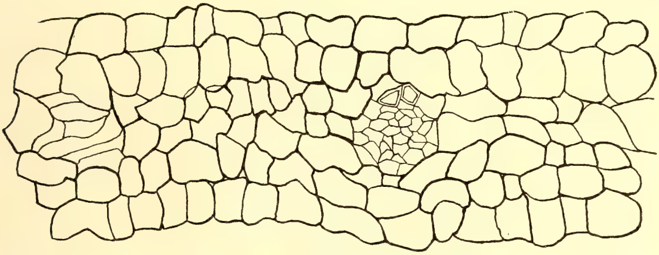

FIG. 3. A cross section of an embryonic bud leaf, magnified 574 times.

IV. VARIATIONS IN THE APPEARANCE OF THE GALL

Here it should be noted that, although superficially the galls appear very similar, a closer examination reveals many variations in the general size, form, number of hairs, and in convolutions. These variations are mainly due to the locality on the leaf selected by the insect, as a gall started on a primary vein is quite different from one started between the veins. A primary vein in a very young leaf is made up of two epidermal layers with a large number of unicellular and a few multicellular hairs, which arise from both layers, a mass of parenchyma cells which make up a large part of the vein, surrounding the vascular elements proper and later on becoming thick-walled supporting cells; the xylem consists of several vessels usually having close and loosely woven spiral thickenings, while the phloem consists of a few (about 30), elongated sieve cells. One of the striking features with reference to the arrangement of galls on the leaf is the fact that they so frequently occur along the veins, especially along the largest veins. The mouth of the gall, which is the hairy opening on the upper surface of the leaf, usually is not circular but has a longer diameter parallel to the axis of the vein. This seems to be due to the fact that the insect places herself with her longitudinal axis parallel to that of the vein. Figure 2 shows the insect in this position, although she is

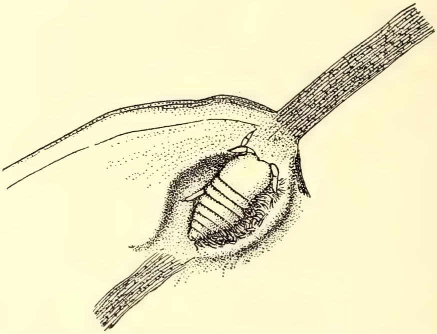

FIG. 4. A nymph attacking a primary vein, resting in the depression.

partly contracted away from the surface. Text-figure 4, however, leaves no doubt concerning her orientation, the insect being shown with her longitudinal axis parallel to the direction of the primary vein, which she is vigorously attacking.

The growth of the gall keeps pace with that of the young leaf. Should anything retard the growth of the leaf, that of the developing gall is correspondingly inhibited. Likewise, should anything happen to the insect, the gall ceases to develop. This has been observed by

Riley (24) and by Cornu (8). Successive daily measurements of the growth of galls shows that 12 to 15 days are necessary for complete development.

V. Histological Development of the Gall

During the winter of 1913, cuttings from a *Vitis vulpina* vine were grown in the greenhouse, and in the following spring, when the first galls appeared out of doors, some of the nymphs were taken out of a gall and placed on young leaves of cuttings growing in the greenhouse. Twenty-four to forty-eight hours later, the first signs of gall formation appeared. The nymph, which had been placed on a bud leaf, as the bud was opening, had located itself on a primary vein, and as the young leaf grew free from the bud scales and opened so that its whole upper surface became visible, the insect was seen in a shallow depression in which it was resting. This marks the first outward sign of gall formation. On the upper surface of the young leaf the gall appears as a shallow depression measuring about 0.5 mm., in depth and in width, the margin of which shows a growth of fine upright hairs, while the under surface of the gall shows a corresponding convexity as compared with the rest of the lower surface.

Text-figure 4 shows an early stage of gall formation such as described above. The figure was drawn under a binocular microscope while the leaf and insect were still living, and shows the insect at work on a vein, lying in the depression and surrounded by the hairs, both of which are the results of her labor. Ráthay (23) gives good colored drawings of the outside appearance of these early stages of gall formation.

In order to follow the histological development of the gall, different stages were collected, fixed in various dilutions of Flemming's, Carnoy's and other fixatives, imbedded in paraffin and subjected to various stains. Nearly all material was cut so as to give a serial arrangement of sections.

The first sign of gall formation in 1914 did not appear until late May. Opening buds from any region of a vine showing galls on the expanded leaves were brought into the laboratory and examined for signs of the grape-vine Phylloxera. Fortunately, I was able to find very early stages in great abundance. Figure 1 shows a cross section of a gall which is not more than twenty-four hours old; the hairs of the upper and lower epidermis of the leaf adjoining the midrib are the results of the first twenty-four hours of insect attack.

Concerning the histological structure and development of the gall, brief descriptions are given by Cook (4), Pantanelli (22), and Cornu (8 and 9). As already pointed out, gall formation starts on the young bud leaf, where the insect usually places herself along a primary vein, either directly on top of the vein or directly alongside of it, and an abnormally large number of unicellular and multicellular hairs, grow up around her from the upper epidermis. The lower epidermis likewise feels the stimulus and the abnormal production of similar hairs is started. The tissue beneath the insect appears sunken so that a basin-like cavity is produced on the upper surface of the leaf, in which the insect lies. Figure 2 shows the insect drawn away from the hollow in which it was resting, but a portion of its proboscis is still seen piercing the tissue of the vein.

The apparent sinking in of the upper tissues of the leaf is due to a partial collapse of the young mesophyll tissue. Figure 9 shows a cross section of a young bud leaf. The proboscis of the insect is seen piercing through the epidermal cells, and appears broken off from the rest of the insect, which is drawn away from the leaf. The proboscis, curved around and reaching to the phloem of the vein, was followed in three successive sections, only one of which is shown. The narrowness of the leaf where the setae of the insect are projecting, and the papillate projections bordering the hollow are worthy of note. The constricted portion measures 72μ as compared to the normal unconstricted portion, which measures 96 μ. Comparison of the cells in the two regions shows that this difference in size between the constricted portion and the unconstricted portion is due to the difference in size of the mesophyll which measures 40 μ at the constricted part, where the proboscis protrudes, as compared with the normal unconstricted mesophyll which measures 68 microns. Thus we see that the first twenty-four hours of insect attack brings forth a decrease in size of the portion attacked, accompanied by the production of hairs around the constricted portion, which is a hypertrophic effect. Reasons for these developments will be discussed later.

Following the histological development of the gall, we find after three to four days of insect attack, that rapid proliferation of the underside of the leaf begins to take place, while the tissue of the upper side shows a slight enlargement of the palisade cells and of the upper epidermis, perpendicular to the surface of the leaf. Figure 3 and higher magnifications of the central part of the same section in figure 4 show the rapid proliferation of the mesophyll of the lower half of the leaf, together with slightly enlarged palisade cells, which only show this enlargement as they distance the proboscis. These two figures are especially noteworthy since they show the proboscis seemingly at work, and extending through the whole width of the leaf. So far as I know, they are the only figures of their kind which have yet been published. The insect is shown resting in a rather deep cavity instead of the shallow depression as it did after twenty-four hours of attack. During this three to four days of insect attack the young bud leaf has had an opportunity to unfold, being two to four times the size which it had at the time the insect first started its attack. It is necessary to bear in mind that the pouch-like form of the gall, which is beginning to manifest itself at this age, not only represents an excessive local growth, but also represents a change in the direction of growth of the tissue attacked. Instead of taking the normal direction of growth, the portion of the leaf beneath the insect grows downward and takes the form of a pouch. This growth downwards, is accentuated by the fact that the lower half of the leaf which does most of the proliferating, grows downward, in the path of least resistance, while the upper half enlarges slightly, without cell divisions.

It is a peculiar fact that the direction of growth in this gall, as in all other galls where the gall producer is situated at one side of a plant organ, is always away from the insect, opposite to the direction of the application of the stimulus resulting in a sort of negative tropism. Küster (12) says: "The side growing most in a leaf gall is always the one away from the gall animal, so that in this rolling of the infected leaf area, the gall animal comes to lie within the cavity thus produced." However, in this gall, although most of the hyperplastic tissue making up the gall comes from the lower half of the leaf, lying beneath the insect, the upper half of the leaf, which goes to make up the sides of the cavity, grows extensively upward. Pantanelli (22) describes this proliferation of the upper epidermis and palisade cells and points to the fact that growth due to the excessive proliferation of these cells finally forms the "lips" of the gall and almost completely encloses the insect.

The striking histological peculiarity of figures 3 and 4, representing 3 to 4 days of insect attack, is not the excessive growths of the lower half of the leaf, since such growths have been described for other galls, but the *lack* of proliferation of both the upper and lower halves of the leaf in the portion immediately around the proboscis, which makes this part appear as a narrow neck between two masses of hyperplastic growths. This is a development which has not yet been described for other galls.

Other features which are worthy of note, at this stage of gall development are represented in figure 7. This figure, which is a highly magnified view compared with the other plate figures, shows a cross section of gall tissue taken from the portion below the insect. Starting from the top, which lined the base of the insect cavity, we see three layers of elongated cells, representing the upper epidermis, the palisade layer, and a layer of irregular mesophyll. As compared with corresponding normal leaf tissue, these cells show marked hypertrophic growth. The walls of these elongated cells, especially the walls running parallel to the leaf surface, are very much thickened in some places and entirely lacking in other places of each individual cell. In some cells, all that is left of the walls are narrow threads, which often show a reticulate arrangement and stain red with safranin. Such cell-wall features are as rare in galls as in any other pathological structures. Weidel (31) reports a dissolution of walls in the larval chambers of Cynipid galls and P. Magnus (19) finds a sieve-like dissolution of the cell walls produced by a Urophlyctis, which he figures. This latter case is more comparable to figure 7 since Weidel reports dissolution of whole walls in his insect galls. It seems that the cells shown in this figure might possibly assume the characters of tracheal elements, similar to that found by Küster (12) in leaf callus of Cattleya. In the outer cells of this callus, he found reticulate thickenings, which in the lower part of the cell are only narrow meshes between single thickened bands, while in the upper part the bands are usually flatter and somctimes partially interrupted. As translated by Dorrance (12), he says: "This case is of special interest since, aside from tyloses, it is the only one known to me in which hypertrophic growth, incited by a wound stimulus, is combined with the formation of a special kind of wall thickening." In the Phylloxera gall we likewise have a thickening and a partial dissolution of hypertrophied cells, giving the appearance of tracheal formations as seen in wound callus of leaves, described by Küster. This reticulate structure is only seen in younger galls, due to the fact that as the gall reaches maturity the cells at the base of the insect cavity collapse and their individuality is lost. These are the most noteworthy histological developments seen at the end of three to four days of insect attack.

At the end of five to six days of cecidial development, the cells of the palisade layer at the base of the insect cavity can be distinguished from the rest of the mesophyll, while at the sides of the cavity their identity is lost and they assume the characters of rapidly dividing thin-walled parenchyma cells, sometimes enlarging enormously and becoming isodiametric or irregularly ovoid in shape. The rest of the mesophyll below the bottom of the cavity, except in the region of the proboscis, continues to proliferate enormously into a mass of thin-walled cells. As to the epidermal cells, some of them enlarge in the plane perpendicular to the surface of the leaf, as was shown in figure 7 for the earlier gall development, but at the upper part of the cavity they give rise to a large number of multicellular hairs. The lower epidermis likewise produces many such hairs.

The daily growth in size of the gall is very marked. In twenty-four hours it may increase 0.3 mm., in its long axis, so that proliferation and enlargement is very rapid. At the end of twelve to fifteen days, when the gall reaches maturity and the normal leaf has attained its maximum size, a section through the side of the gall, cut perpendicularly to the leaf surface, reveals an enormous mass of thin-walled, partly empty parenchyma cells, some of which are greatly elongated. This is the most striking feature in the mature gall.

The histological structure of the mature gall is as follows: Starting with the lower epidermis, we note the small number of stomata, while the epidermal cells show very little cuticular development. The epidermal cells are usually smaller or the same in size as the normal epidermal cells, although they sometimes appear elongated and narrow, running parallel to the surface of the gall. The corrugations or striations of the cutinized layer of the normal leaf are absent in the epidermal cells of the gall, except where it bounds vein parenchyma, in which case the striations are very evident. The mesophyll is made up of a mass of cells, many of which are rather undersized, as compared with normal mesophyll cells, while others are usually elongated perpendicularly to the surface of the gall as noted by Cook (4). However, they sometimes turn sharply and run parallel to the surface of the gall. This condition is brought out in figure 5. The arrow is pointing to groups of such elongated cells which are bent at right angles to the surface of the gall. But the striking feature in this huge mass of tissue is its compactness and the total absence of air spaces. As Cook points out, the palisade cells cannot be distinguished; at the bottom of the cavity they are found in a more or less collapsed condition while at the sides they are usually not to be distinguished from those of the mesophyll. The vascular elements have also been changed from their normal arrangement. Although in total amount they are not noticeably increased, they are frequently scattered and twisted into separate small groups. Figure 6 shows how the xylem and phloem from one vein are separated and scattered by wedges of parenchyma cells, which usually show thickened walls. Compare this scattered mass of vein tissue in the center of the gall with a normal vein, marked N, in the same figure. Sometimes the vascular elements in the gall take the form of cylindrical or circular masses of cells which reminds one of the ball-like groups of tracheids that Küster (12) describes in callus tissue. What has been said for the lower epidermis can also be said for the upper which lines the cavity of the gall; besides that, at the base of the cavity the epidermal cells as well as several layers of mesophyll are totally or partially collapsed.

VI. Chemical Constituents of the Phylloxera Gall

Figure 8 is a photomicrograph of a section cut from the center of a mature gall. It represents a very low magnification as compared with the other plate figures. Some of the eggs and nymphs are still visible inside the cavity of the gall. This section also gives a good view of the so-called "nutritive zone" of the gall, described by Cook (4). It is represented by the very dark mass of cells below the cavity of the gall. These cells are filled with tannin, crystals of various forms, starch grains and other substances; because of this, this portion of the gall stains heavily. Pantanelli (22) has made a chemical analysis of this Phylloxera leaf gall and finds that gall-bearing leaves contain more total organic nitrogen and more proteic nitrogen than normal leaves; the ash content is lower in lime, iron and magnesium in the gall-bearing leaves. He finds an abundance of starch, albumin, fat and phosphates in the nutritive zone.

Molliard (20) made a chemical analysis of two leaf galls of the elm, both produced by plant lice, *Schizoneura lanuginosa* Hartig and *Tetraneura ulmi* De Geer. Comparing the same weight of gall tissue with normal leaf tissue he finds that galls contain a greater percentage of water, a smaller percentage of ash, a total absence of sucrose, a greater percentage of reducing sugars, four times as much tannin, a smaller total nitrogen content but a greater percentage of soluble nitrogen. His results are quite similar to those obtained by Pantanelli with the exception of the total nitrogen content. Molliard thus points out that his results as well as those of Pantanelli, and Paris and Trotter, indicate an increase in simple substances in galls. Furthermore Molliard finds an enhancement of respiration, an increase in the oxidases laccase and tyrosinase, and in free acid. He concludes that the insects inject into the plant tissue certain enzymes, which may explain the presence of a large amount of simple substances.

VII. Discussion of the Stimuli Producing Galls

In stimulated structures generally, we may find an increase in enzymes, as Czapek (11) demonstrated for geotropically stimulated roots, where he found an increase in oxidases, especially tyrosinase. Von Schrenk (30) likewise found an increase in oxidizing enzymes in intumescences produced by sprays of various copper salts on cauliflower leaves. It seems, therefore, that the presence of a large amount of these enzymes in galls, especially in the Phylloxera gall, does not necessarily mean that they have been injected into the attacked tissue, but on the contrary, from our knowledge of enzymes in other stimulated structures, there seems to be good reason to assume that such is not the case.

Before going further into a discussion of stimuli producing galls, it will be best to discuss the early development of the Phylloxera gall. We noted in figure 9, which represents gall formation at the end of the first twenty-four hours of insect attack, that the portion of the leaf beneath the insect, in the vicinity of the proboscis, is constricted and measures 72 μ as compared to the normal width of the leaf, which measures 96 μ. Furthermore, it was shown that this difference in size between the constricted and the unconstricted, normal portion, was due to the decrease in size of the mesophyll of the attacked part. Besides this, we noted the beginning of abnormal hair production at the borders of the depression.

How has the insect brought about the decrease in size of the attacked mesophyll and the consequent depression? Is it due to sucking out the contents and a consequent decrease in size of the portion attacked? This seems the most reasonable explanation. Although a few of the upper epidermal and mesophyll cells are killed (I have never found more than two or three epidermal and two mesophyll cells killed), by the action of the proboscis, the hollow is not the result of their death and a subsequent sinking in of the neighboring tissue.

Employing the technique used by Barber (2 and 3), a considerable number of fine, capillary glass tubes were made measuring from 5 to 20 μ in diameter and around 0.5 mm., in length. About 25 of these tubes were stuck into very young vine leaves and allowed to remain there until the leaves had grown to a fair size. Several punctures in small leaf areas were made in some cases and a circle of india ink was drawn around each area to indicate the place of operation. Young leaves punctured in this manner were also permitted to grow to a fair size. Sections were then made of the wounded leaf areas, and while microscopic examination showed dead cells in the punctured regions, no depressions were found around the point of injury. These experiments add weight to the belief that the depression in the vine leaf below the insect is not due simply to a puncturing by the insect's proboscis. Furthermore, in figure 9, it is seen that there is a depression not only of the upper leaf tissue but also of the lower, where the proboscis has not gone through and has not killed any of the lower epidermal cells.

It occurred to the writer that the force exerted by the insect's body pushing against, and weighing down upon a delicate, embryonic leaf, might have something to do with the formation of the depression. The furrows made by twiners on growing plants would perhaps be an example of the effect of such a force. Moreover Molliard (21) has noted the fact that a pressure exerted upon the surface of a growing portion of a plant may cause a depression at the point of contact, and an increase in growth, a hyperplasia, in portions adjoining the depression.

To test the effect of a force comparable to that exerted by the body of the insect, fine glass needle points, made in the same manner as those described above, measuring 5 to 20 microns in thickness and several millimeters in length, were held over a very small flame, so that the heated end coiled up and consolidated into a small glass knob. The tubes thus treated appeared as very small, round-headed, glass pins. These were so stuck into the young vine leaves that the heads pressed against the surface of the leaves. The pressure of the pin head was to take the place of that of the insect body. After the leaves had grown to a considerable size, microscopic examination of the injured areas showed dead cells which the pins had pierced. A slight depression was noticeable where the head of the glass pin had pressed against the leaf surface, but nothing was obtained resembling the insect cavity, with its fringe of hairs.

It seems, therefore, that, at the beginning of its work, the insect by its sucking has caused a partial collapse of the tissue attacked, or at least a cessation of its growth, resulting in a sunken area or hollow in which the insect rests. What is the cause of the enlargement of the epidermal cells and why do they divide to produce multicellular hairs?

The production of hairs occurs quite commonly in gall formation. Erineum galls are almost entirely given over to the formation of hairs. As to the abnormal production of hairs other than in gall formation, Haberlandt (13) caused groups of colorless hairs to be formed by destroying transient glandular hairs of *Conocephalus ovatus* and *C. suaveolens*. The glandular hairs functioned in eliminating water and their removal, according to Haberlandt, resulting in a surplus water supply, caused the formation of intumescences consisting of bunches of hairs. Küster (12) points out the similarity between epidermal leaf hairs of Erineum galls and root hairs. He cites Schwartz as stating that changes in cell turgor may cause abnormally large root hairs. Sorauer (26) noted that wooly tufts were produced on the inner side of the core of the apple, which he assumes were due to an excess of water. This formation, Küster says, resembles callus tissues.

We note that in these cases a pressure stimulus has been put forth by the various investigators, as the initial factor in the abnormal production of hairs. The removal of certain organs or a retardation of a function of certain parts induces an abnormal pressure on adjoining parts which results in increased growth.

As stated above and shown in figure 9, the insect causes a decrease in size of the mesophyll cells in the region where the proboscis is at work, a decrease which seems to be due to a retardation in growth, and which makes this part appear as a depression in the leaf. Around the periphery of this depression thus caused, hairs are formed. May it therefore not be assumed that these hairs are the result of the stimuli caused by change in tension and pressure brought about by the partial collapse of the attacked mesophyll? The evidence presented above by various investigators seems to substantiate such an explanation to account for the abnormal production of hairs.

Küster claims that in Erineum galls the stimulus causing the abnormal growth of the epidermal cells "comes from a poison which the gall insects produce, concerning which nothing more is known." This theory, as well as any other *chemical* theory, makes it difficult to explain the production of hairs in the Phylloxera gall. If we assume that a chemical substance is introduced into the leaf by the proboscis, then we must also assume that this substance, starting from the proboscis as a center, should diffuse or osmose equally in all directions; or, if on the other hand we assume that the substance given off by the insect is not readily diffusible in the tissues but that it initiates certain stimuli, which can be felt some distance from the initiating center, then we must also assume that changes or responses induced by these stimuli should appear equally distributed in all directions. But in the Phylloxera gall, as I have pointed out, the response is rather unequal, none or few hairs are produced in the depression, but they are produced always with perfect regularity at the edge of the depression. Does not the character of the response seem to point to a mechanical stimulus rather than to a chemical one? The mechanical stimulus would appear to be in the nature of a change in tension or pressure, which stimulates growth just where the tension would be greatest, *i. e.*, at the borders of the depression. Küster carefully points out that such forces, besides others, are at play in callus formations. Cornu (9), who has made a very careful study of the root gall caused by this plant louse, and whose work has been either overlooked or disregarded by cecidiologists, likewise concludes that the sucking of the insect on the root, resulting in cessation of growth of the attacked part, produces tensions which dilate other elements not hindered in their growth.

In the description of the histological development of the gall when it is three to four days old, we noted that the upper half of the leaf directly below the insect showed no proliferation, while the under half, on both sides of the narrow portion, proliferated very abundantly. The lack of growth of the upper half of the leaf, which is directly below the insect, is not a specific character of this gall only but, as Cosens (10) points out, this phenomenon is the usual occurrence in the simpler galls, in which the stimulus is applied in one direction only, and also exists in the highly complex Neuroterus gall described by Weidel (31). This latter author believes that mechanical stimuli are the factors in the production of even the most highly developed Hymenopterous galls. He questions Beyerinck's (1) hypothesis of chemical stimulation and asks why is it that the proliferation is more pronounced around the larva, *i. e.*, in tissues some distance away, than in tissues immediately in contact with it? Cosens, who believes that gall producers secrete enzymes which bring about cecidial formation, answers this question by saying that it seems likely that the enzyme content requires a certain degree of concentration in order to exhibit its maximum activity, and that immediately in contact with the larvae the enzymes do not possess the requisite degree of dilution to cause the greatest stimulation.

In the Phylloxera galls, as noted, the tissue next to the nymph shows no increase in the number of cells, and furthermore the tissue immediately around the proboscis showed no proliferation. If this insect introduces any diastatic enzymes, as Cosens believes, they must be introduced as salivary secretions by means of the proboscis, since secretions from such structures as cenocytes, as Rössig (25) describes, or Malphigian tubules, as Triggerson (28) describes, of the insect body, would be made in this case on exposed leaf surfaces with very little chance of entering the leaf. I have sprayed solutions of diastases on young vine leaves and observed no indication that it entered the leaf. According to Cosens's hypothesis the area immediately around the proboscis would not grow much, because the concentration of the enzymes would be too great, but further away, where the concentration of the enzymes would be less, growth would be greater. It is difficult on this basis to explain the phenomena under consideration. Cosens assumes that these enzymes are readily diffusible, an assumption which is not supported by investigation. Magnus (18) says that material coming from the insect which may play a part in gall formation does not have to be readily diffusible, but Küster (17) says: "Das einzige, was wir von den Eigenschaften der von den Cecidozoen gelieferten Stoffe wissen, ist, dass sie wasserlöslich sind und auf dem Wege der Diffusion durch Zahlreiche Zellschichten im Körper der Wirtspflanze sich verbreiten können."

Cosens performed several experiments, in which he placed the larvae of *Amphibolips confluens* on starch solutions and after a time obtained a test for sugar. From this he concludes that the Cynipid larvae excrete an enzyme capable of changing starch to sugar. He states that at present there is a tendency to ascribe the stimulating agent in gall production to enzymatic action and he holds that this is a safe working hypothesis. He is careful to point out that only in the Cynipidae, referring to his own diastatic experiments, do we have experimental evidence for this. Magnus (18) says, not only the larvae of the Cynipidae give off diastatic and proteolytic enzymes, but larvae of the Diptera do the same thing. He describes an experiment in which larvae of *Dasyneura* (*Perrisia*) *terminalis* were placed on a starch-gelatin medium and remained alive for a long time. After twenty-four hours, the starch around them had been dissolved in a ring, showing a diastatic action, and similarly the gelatin had been dissolved by proteolytic enzymes. However, he does not conclude from this experiment that gall production may be traced to these enzymes, and very critically he says: "Erscheint also auch immerhin die Mitwirkung der vom Gallentier ausgeschiedenen proteolytischen Enzyme bei der Gallenbildung möglich, ist hierfür bisher kein Beweis erbracht, vielmehr gaben alle Versuche, mit proteolytischen Enzymen die Pflanzengewebe in andere Entwicklungsbahnen zu lenken, negative Resultate."

Performing experiments along the lines described above, I extracted hundreds of Phylloxera nymphs from galls and placed them on a starch solution. I obtained sugar tests in a number of such experiments, while checks gave no tests. I am not sure, however, that I did not introduce wild yeasts or other micro-organisms along with the nymphs. However that may be, when I sprayed young vine leaves with a fine spray of a watery solution in which the nymphs had been deposited, my results were negative. Injections of this solution into the leaves with fine glass tubes all gave negative results. Since only a small number of injections were tried, the results may not be conclusive.

Magnus (18), who has given us an excellent summary of the theories concerning the etiology of gall formation, concludes that the stimuli are not enzymatic as Beyerinck, Cosens, Molliard and others believe, or specific poisons, chemomorphs, as Küster and others maintain, but substances which inhibit the action of enzymes, substances which may be likened to the anti-enzymes of Czapek, antibodies of serum biologists, or hormones as described by Armstrong.

These substances, Magnus believes, are given off by the insect, or they may be the results of a material exchange between the living cells of parasite and host. They may produce osmotic disturbances, which will affect the nutritive processes of the plant tissue involved, and so give rise to gall production. This is quite hypothetical and as Magnus himself says, no direct evidence has been brought forth in support of the theory.

It is beyond the scope of this paper to go into all the theories put forth to account for gall production. Küster, in his articles and books, and Magnus (18) give thorough, up-to-date accounts of these discussions. Most of these are centered around a "chemical" theory, in which it is supposed that the producer injects some kind of chemical which serves as the stimulus for gall production. These theories are invoked to account for galls produced not only by one special class or order of insect gall producers, but for all gall producers, nematodes, mites, insects, and fungi. Küster, who attempts to classify cecidia on a structural basis, is an exception. He (16) thinks that different kind of stimuli may produce "organoid" galls from those which produce "histoid" galls. He is a firm believer in the "chemical" theory to account, at least, for his "histoid" galls. He pushes this theory a step further and says that certain kinds of chemicals, "chemomorphs," produce certain kinds of galls.

If any such chemical substances are injected into the vine leaf by the Phylloxera insect, it seems to me that their effect would be almost negligible as compared with the effect of a continuous sucking action for fifteen days at one fixed point, as far as *initial* stimuli produced by the insects are concerned. Here it should be pointed out that I have interested myself in the *initial* stimulus only. Undoubtedly the final stimuli for growth, in galls as well as for ordinary normal growth, are chemical stimuli; but, as Küster says, between the initial cause and the final effect there probably intervenes a "chain of stimuli." Thus wounding may release certain chemical stimuli which will bring forth callus formation [see Haberlandt (14)], but the initial stimulus is the wound.

Cornu (8 and 9), whose thorough, painstaking study of the root gall of *Phylloxera vastatrix* strikes one as being authoritative, concludes that the insect does not inject any poisons or other chemicals which are the stimuli for gall production. He gives the following reasons for this conclusion. First: the attacked rootlets first are made to take the form of hooked swellings and then they die. If a poison is injected by the insect which produces first a swelling, why, he asks, does the same substance later on stop growth? Second: when roots of the vine attain a diameter of more than 3 or 4 mm., no swellings are produced, although a considerable number of Phylloxera are often seen on such roots. They are grouped or aligned in the bark cracks, alongside of each other, and if they give off an acrid, irritating fluid, they ought, united in a mass, to produce considerable disturbance and a proliferation of the elements of the cortical tissue. Their effect, however, is very feeble. Cornu says if the argument against this is offered, that the bark cells cannot respond because they are older cells, it may be pointed out that each year the old bark is exfoliated by means of a new suberized layer, coming from the embryonic tissue, the cork cambium. The effect produced is a hypertrophy and a coloring of certain gum reservoirs. Third: the galls of the stem and tendril are produced by a portion of the cortical tissue around the Phylloxera and not immediately below it. He says, it is not a local excess of acrid liquid concentrated at one point, which stops the formation of new tissues; for, in the cells more or less distant from this point, where the effect of this excess should be less effective, the hypertrophy should still manifest itself. This it does not do. Directly below the insect beneath a few layers of cells, is the generative zone, as Cornu points out, referring to the cambium layer of the stem or tendril. This zone does not produce any new growths. The swelling of the root is not produced under the insect, at the point punctured by it and where it produces a depression, but in the region farthest away, which makes up the hook form of the swellings. Fourth: if there has been a chemical irritant introduced, it should manifest itself by a swelling immediately in contact with the insect, for even after an attack of only several hours, there is found, around the point which has been occupied, an obvious depression. Cornu came to the conclusion that the puncturing by the proboscis together with the absorption of cell contents is sufficient to explain cellular segmentation and production of new tissue. To prove this contention and to show that irritating liquid had nothing to do with gall formation, Cornu injected into roots, stems, and leaves, 25 per cent. solution of acetic acid and 10 per cent. solution of sulphuric acid. Although in one or two cases he obtained swellings, he got no depressions, so that he is satisfied that gall production is the result primarily of the sucking by the insect. His experiments on this point are very scanty and some of his reasons against chemical stimulation are open to question. Cornu noted that the sucking and puncturing stops the development of certain cells and as a result, he says, tensions are set up which cause neighboring cells to become dilated up to a certain size and then to divide. On the leaf the tensions occur on the underside, so the growth is in that direction, while on the rootlets the tension is felt on the sides, so the growth is at the sides of the insect.

Cook (5) studied the mouth parts of several Hemiptera gall producers, among which was *Phylloxera vastatrix.* He says: "So far as I have been able to determine, the insects do not remain attached to any one point for a great length of time." From this he concludes that the modification of plant tissue to form the gall is purely mechanical, being a continuous effort on the part of the plant to heal the wound produced by the repeated puncturing of the cells by the insect. Cornu (8) however, finds that the insect in the *Phylloxera vastatrix* leaf and root gall remains immovable, and fixed by its proboscis to the bottom of the cavity. My observations substantiate those of Cornu in that the insect, as soon as it fixes itself on the upper epidermis, seems to remain fixed for at least a considerable period. A proof for this is the frequency with which I have found the proboscis in the tissues in many of my prepared slides. But the immobility of the insect manifests itself in several other ways. Text-figure 2 shows how closely the insect fits into the cavity, so close in fact, that it could hardly move around in it, and in this case the insect has remained attached to the leaf from twenty-four to forty-eight hours. Again, cross sections of young and old galls show the proboscis usually at one fixed place, which place may be marked by a greater depth of the insect cavity, and by the broken-up epidermal and mesophyll cells, which are very few and are only found where the proboscis has penetrated. We may therefore conclude that Cook's hypothesis of a mechanical stimulus playing the part in gall production is not founded on observed facts, since the insect remains fixed and does very little puncturing.

The following facts stand out markedly in the formation of the Phylloxera gall: The young nymph attaches itself to the upper surface of a bud leaf. Within twenty-four hours, or less, the attacked portion shows a depression, the edge of which is bordered by hairs, while the growth of the tissue in which the proboscis is working is arrested. Within thirty-six hours, the lower half of the leaf tissue, which is situated some distance from the portion attacked, begins to enlarge rapidly, giving rise to enormous hyperplastic growths. While the lower side of the attacked leaf has been growing enormously, the upper portion of the leaf directly beneath the insect, which shows at first an elongation of epidermal and palisade cells, does not increase in the number of cells. This portion becomes the bottom of the cavity, while the upper portion of the leaf, which surrounds the insect, greatly proliferates, grows upward and becomes the sides of the cavity. As the leaf grows, the growth of the gall proceeds, and at the end of twelve to fifteen days when the leaf is almost fully expanded, the gall has attained its full size and encloses the cecidial producer, which has increased in volume many times. The gall also contains several hundred eggs which the insect has laid. Text-figure 5 shows such a gall, partially cut open. The one thing that is definitely known concerning the work of the insect is that it has sucked for fifteen days at one very small area, and that it has obtained enough nutriment to feed itself and to enable it to produce several hundred eggs. This sucking action suggests itself as the initial stimulus in gall production. It appears to the writer that the disturbances which such an action would produce, such as the lowering of tensions at one part and the increase in another, the change in osmotic relationships necessary to counterbalance the withdrawal of cell contents, are factors which are sufficient to account for the localized abnormal growth.

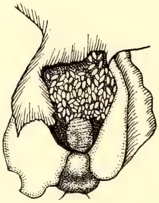

FIG. 5. An opened gall. Showing the gall producer and her eggs filling the cavity. (Note the narrowness of the gall where the insect is sucking.) Magnified 5 times.

Von Schrenk (30) produced intumescences on cauliflower leaves by spraying them with various copper salts. I have likewise produced these intumescences both on cabbage and on cauliflower. Figure 10 shows a cross section of a cabbage leaf with a large intumescence projecting from the lower side of the leaf. The leaf was sprayed with a very fine spray, only on the under side, with a solution of ammonium copper carbonate made up of 4.5 cc. ammonia, 0.5 g. copper carbonate and 750 cc. of distilled water. These hyperplastic growths may be made to appear on the upper or lower surface of the leaf, depending on whether the leaf is sprayed on the upper or lower side.

In no case are depressions or cavities produced at the point of contact between the leaf tissue and the chemical applied. The growth response is always in the mesophyll tissue immediately below the point where the spray was applied and not on the side furthest away from the application of the spray. Sprays with commercial diastases on young cauliflower leaves likewise produced intumescences, but I feel that this latter experiment was not done on a large enough scale to warrant any conclusions. It is possible that other substances in the commercial diastase were factors in the production of the intumescences. If any conclusion may be drawn from these experiments it is that excessive growth takes place in those cells in which the applied chemicals are at their greatest concentration, and not at a distance from the center of application, where the concentration would be less.

If we compare these artificially produced intumescences with the Phylloxera gall which has been described above, it will be seen that in the intumescences produced by the application of chemicals, the place of application is the place of excessive growth, and in the Phylloxera the place of application is the place of hindrance of growth. From these experiments the burden of proof becomes more difficult for those who adopt the "chemical" theory of gall production for sucking insects. This is especially true in the case of the Phylloxera gall.

VIII. Summary

1. The *Phylloxera vastatrix* leaf gall starts to develop on embryonic bud leaves. In twenty-four hours the insect produces a depression at the periphery of which hairs are formed on the upper surface of the leaf. The depression is due to a lessened growth of the attacked mesophyll.

2. After three to four days of insect attack the lower half of the leaf tissue which surrounds the portion in which the proboscis is inserted has proliferated enormously. The whole thickness of the leaf in the region immediately around the proboscis shows no proliferation. That portion of the leaf which is beneath the insect does not proliferate, but the upper half at the sides of the insect grows upwards and forms the walls of a large insect cavity. Upper epidermal cells and several

layers of mesophyll cells in the portion of the gall below the insect, show peculiar thickening and dissolution of their cell walls.

3. Gall development depends upon leaf development; when the leaf reaches its maximum size, after twelve to fifteen days of development, the gall becomes mature.

4. A mature gall shows but slight cuticular development and very few stomata. The mesophyll is a huge mass of compact, thin-walled, partly empty cells, some of which are undersized, and others enormously elongated, the vascular elements are scattered by wedges of parenchyma cells. Many unicellular and multicellular hairs grow out from the gall.

5. Chemical work on this gall shows it to be a structure in which anabolic processes are lacking, and in which large amounts of simple sugars and simple proteins are present.

6. The development of this gall does not seem to support the theory that the insect injects some chemical into the leaf which causes gall formation.

7. Intumescences produced by chemical sprays result from entirely different kinds of hyperplastic responses than hyperplastic gall growth.

8. The investigation establishes the fact that the proboscis may pass through the entire thickness of the leaf.

9. The insect remains fixed, and that portion of the leaf in which the proboscis is fixed is marked by lack of growth as compared with the huge outgrowths which surround it.

10. The continuous sucking action by the insect at one fixed point for fifteen days is believed to be the initial stimulus for gall development.

The work on this paper was done in the Botanical Laboratory of the University of Wisconsin. My appreciation is due to Professor J. B. Overton, under whose direction the work was done, and to Professor W. S. Marshall for helpful suggestions and for the use of his private library.

U. S. National Museum

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DESCRIPTION OF PLATES XIV AND XV

All the figures are microphotographs taken with various Leitz and Zeiss objectives and eyepieces.

FIG. 1. A cross section of an embryonic bud leaf 24 hours after insect attack showing the formation of hairs on the upper and lower leaf surfaces. On the upper surface the hairs are produced only at the sides of the insect. Magnified 102 ×.

FIG. 2. A longitudinal section of a primary vein of a bud leaf. The insect is partially withdrawn from her normal position, but the end of her proboscis is still projected into the upper part of the vein. The beginning of hair formation on the upper surface of leaf and vein may be seen on both sides of the insect. Magnified 127.5 ×.

FIG. 3. A cross section through the center of a three to four day old gall. Showing proboscis protruding through the entire width of the leaf, and showing the narrowness of the gall where the proboscis is working. Magnified 119 ×.

FIG. 4. A higher magnification of the central part of Fig. 3. Magnified 212.5 ×.

FIG. 5. A cross section of a mature gall showing among other things bent parenchyma cells. Magnified 14.5 ×.

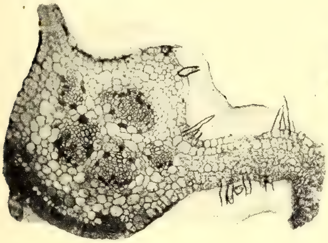
FIG. 6. A cross section near the center of a mature gall showing the mouth of the gall, the scattering of the vascular elements, etc.

FIG. 7. A cross section of three layers of cells immediately below the insect in a three to four day old gall. The thickening and dissolution of the cell walls, giving the appearance of a reticulate structure of tracheae is shown. Magnified 498 ×.

FIG. 8. A cross section through a mature gall showing the nutritive zone, nymphs and eggs in the cavity, etc. Magnified 14 ×.

FIG. 9. A cross section of an embryonic bud leaf, showing the first signs of gall formation, the proboscis protruding from the upper epidermis, the narrowness of the leaf at this point and the beginning of hair formation at the sides of the insect. Magnified 127 ×.

FIG. 10. A cross section of an intumescence, produced on a cabbage leaf by a spray of ammonium copper carbonate. Magnified 53 ×.



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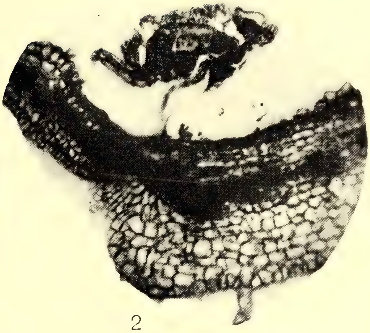

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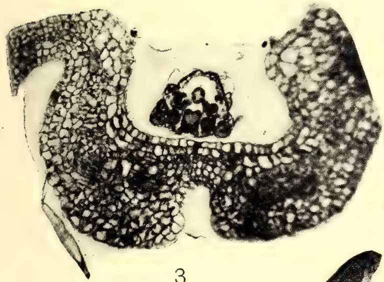

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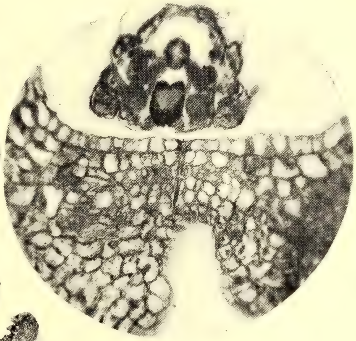

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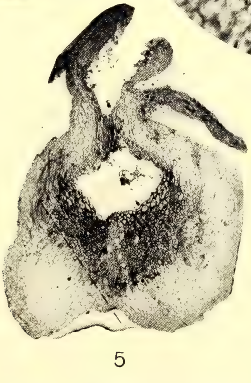

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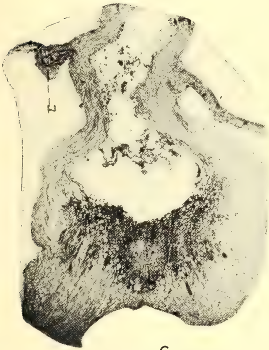
ROSEN: PHYLLOXERA LEAF GALL.



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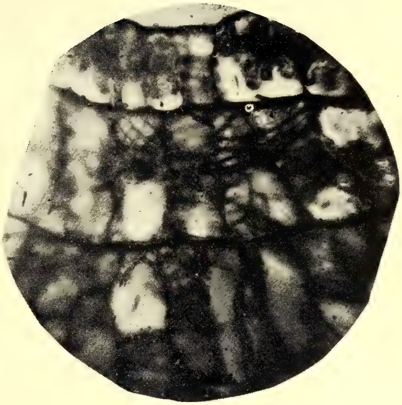

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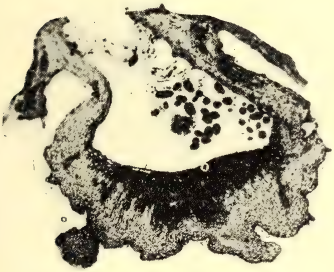

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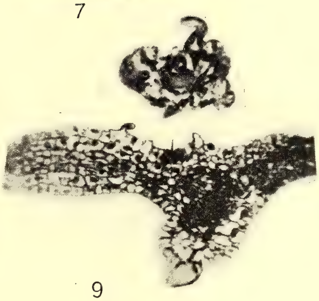

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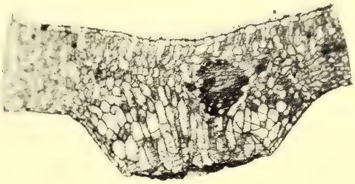

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ROSEN: PHYLLOXERA LEAF GALL.

CORRELATIONS BETWEEN MORPHOLOGICAL CHARACTERS AND THE SACCHARINE CONTENT OF SUGAR BEETS

FREDERICK J. PRITCHARD

In nearly every line of plant or animal improvement, the breeder strives to attain an ideal type which, from study and experience, is known to be correlated with desirable qualities. As long as selection towards an ideal type is confined to characters which bear the desired qualities as constituent elements, as, for instance, size, length and tensile strength of cotton or woolen fiber, this practice appears to be sound. Often, however, in forming the ideal type, a distinction is also made between characters which do not bear the qualities sought, but which are supposed to have a fundamental connection with their development. Before making distinctions between characters of the latter class it would seem desirable to ascertain (1) what the relative merits of the mutually exclusive characters of the organism are with respect to its quality and productiveness and (2) whether highest quality and maximum production are really dependent upon a particular combination of such characters. The present investigation is directed along these lines, but is limited to a study of the sugar beet.

MATERIAL

All the material, except that used in computing the biometrical constants for table II, consisted of five American varieties of sugar beets grown by the Office of Sugar Plant Investigations, at Brookings, South Dakota, in 1910, in co-operation with the South Dakota Experiment Station. About an equal number of beets were taken at random from each variety to compile the data.

The chemical analyses were made by Guy Youngberg, under the direction of James H. Shepard, station chemist.

INVESTIGATION

The correlations which obtain between mutually exclusive characters of the sugar beet plant and its percentage or quantity of sugar seem to depend upon the structural relationship of its different parts. The tissues of the root as shown in cross-section (fig. 1) show a concentric appearance resembling the annual rings of a tree. Wood-zones alternate with zones of parenchyma.[1] The former[2] are richer in sugar than the latter, hence the greater the number of wood-zones and the closer they lie together, the richer the root.

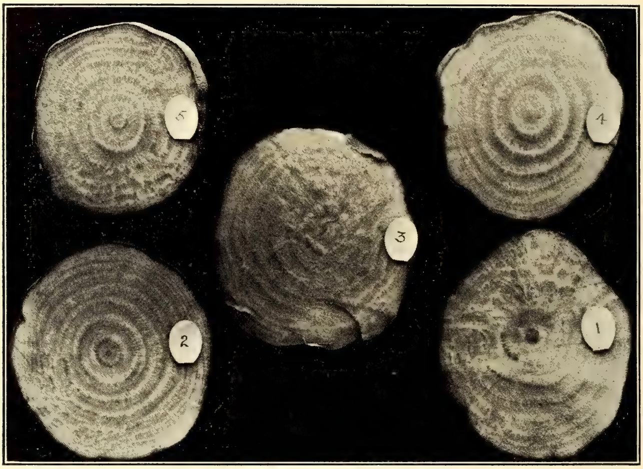

FIG. 1. Cross-sections of sugar beet roots showing wood-zones and parenchyma-zones. (Photograph by Harry B. Shaw.) Sections 2, 4, and 5 are cut from the root; sections 1 and 3 from the lower part of the crown.

The percentage of sugar in the beet depends somewhat upon the size of the root. As small roots usually have as many zones of wood as large roots and relatively less parenchyma, they contain the higher average percentage of sugar. The relationship between percentage of sugar and size of root as found in beets at Brookings in 1910 is shown in table I.

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[1] Bundles from adjacent wood-zones frequently anastomose, but the zones are fairly distinct in cross-section, except in the crown, where they run obliquely.

[2] Samples of 30 roots separated into wood-zones and parenchyma-zones and analyzed separately showed an average difference of 2.6 percent sugar in favor of the wood-zones. This is due to the greater abundance of sugar in the sieve cells and prosenchyma immediately surrounding the bundles.

TABLES I, III-*b* AND IV

Summary of Biometrical Constants

Number of Table	Number of Roots Analyzed	Characters Employed	Mean	Standard Deviation	Coefficient of Variability	Coefficient of Correlation
I	3,784	Percentage of sugar in beet..	17.67± .017	1.59± .012	8.99±.069	
		Weight of individual roots, in grams........	458.52±1.64	149.70±1.16	32.64±.278	−.258±.010
III-*b*	3,784	Percentage of sugar in beet..	17.67± .017	1.59± .012	8.99±.069	
		Quantity of sugar per root in grams........	79.87± .266	23.94± .186	29.97±.263	.005±.011
IV	3,784	Quantity of sugar as per root in grams........	79.87± .266	23.94± .186	29.97±.263	
		Weight of root in grams........	458.52±1.64	149.70±1.16	32.64±.278	.92 ±.0016

The table shows that small roots are richer on the average than larger roots, that the correlation is negative and amounts in this case to —.258. Since correlation varies between 1.00 and —1.00, —.258 represents a fairly large coefficient.

For the purpose of further comparison, similar calculations were made from four groups of beets grown at Fairfield, Washington, and the results summarized in table II.

TABLE II

Correlation between Percentage of Sugar and Weight of Root of Beets Grown at Fairfield, Washington

Group	Year	No. of Roots	Coefficient of Correlation	Percentage of Sugar		Weight of Roots, Ounces	
				Mean	Standard Deviation	Mean	Standard Deviation
a	1907	230	−.284±.041	21.15±.056	1.26±.039	22.39±.311	6.99±.219
b	1909	400	−.499±.025	20.37±.049	1.47±.035	20.70±.025	7.61±.181
c	1910	400	−.257±.031	17.34±.044	1.31±.031	19.00±.290	8.61±.205
d	1910	400	−.253±.032	18.76±.042	1.25±.029	20.71±.213	6.32±.151

These coefficients of correlation are practically the same as those of the preceding table, except for the year 1909. All show a relatively high negative correlation, confirming the general statements of Briem, v. Proskowitz (cited by Fruwirth (1)), and v. Rümker (2), and the statistical results of Harris and Gortner (3)[3]. The regression equations for each of the foregoing tables have been calculated by means of the formula

$$p = \left(p - r\frac{\sigma p}{\sigma w}w\right) + r\frac{\sigma p}{\sigma w}w,$$ [3]

and the relationships expressed in the following graphs (figs. 2 to 6) which also include the empirical means.

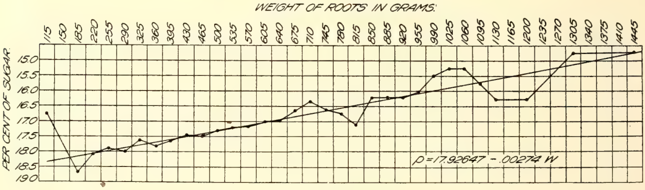

FIG. 2. Relationship between percentage of sugar in the beet and weight of root. (To accompany table I.)

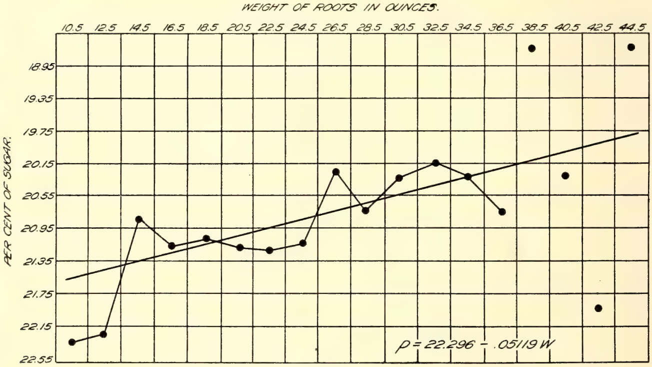

FIG. 3. Relationship between percentage of sugar in the beet and weight of root. (To accompany table II, group *a*.)

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[3] p equals percentage, w equals weight, r equals coefficient of correlation, the sigmas indicate the standard deviations of the two variables and the bars indicate means.

There is a decrease in figure 2 of 35 × .00274 or .0959 percent sugar for each increase of 35 grams in weight. As the weight of roots in the remaining figures (3–6) is expressed in ounces, the regression in percentage of sugar for each unit of weight is somewhat larger than in figure 2 as may be seen in the second member of their respective equations.

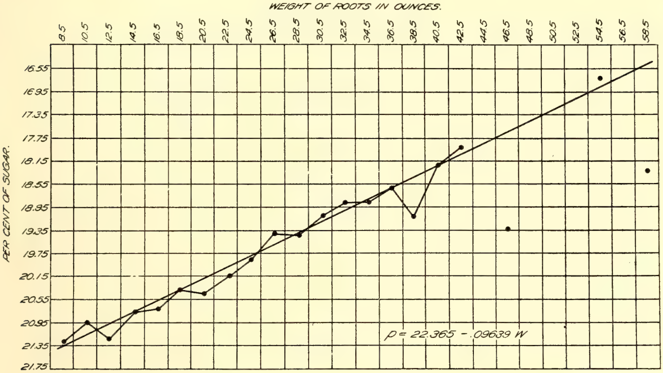

FIG. 4. Relationship between percentage of sugar in the beet and weight of root. (To accompany table II, group *b*.)

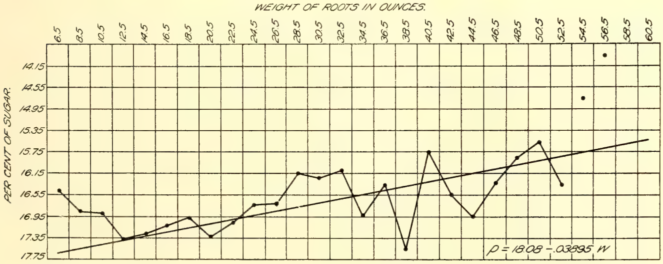

FIG. 5. Relationship between percentage of sugar in the beet and weight of root. (To accompany table II, group *c*.)

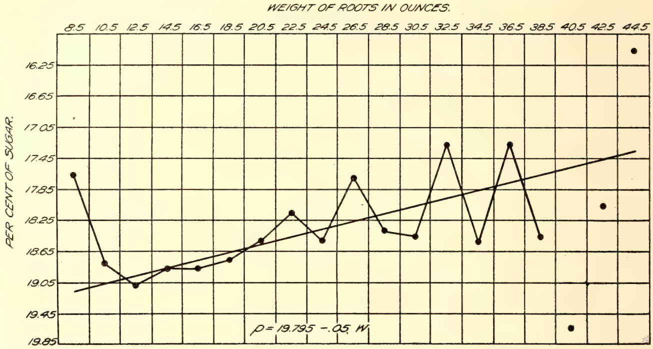

FIG. 6. Relationship between percentage of sugar in the beet and weight of root. (To accompany table II, group *d*.)

The correlation between percentage and quantity[4] of sugar found in beet roots of approximately equal weight is shown in table III *a*.

TABLE III-*a*

Correlation Between Percentage and Quantity of Sugar per Root, in Roots of Approximately Equal Weight

No. of Roots	Weight of Roots in Grams	Percentage of Sugar		Quantity of Sugar		Coefficient of Correlation
		Mean	Standard Deviation	Mean	Standard Deviation	
417	413–447	17.75±.049	1.49±.035	73.86±.221	6.69±.156	.99±.001
371	448–482	17.70±.050	1.44±.035	82.35±.252	7.21±.178	.93±.004
313	483–517	17.40±.057	1.51±.041	87.22±.289	7.59±.205	.96±.003

Although the weight of each group of roots covers a range of 35 grams, the correlation between percentage and quantity of sugar per root is nearly perfect, varying in the calculations from .93 to .99.

[4] As the roots belonged to our breeding material and were to be used for growing seed they were not topped before weighing. The quantity of sugar was calculated on the total weight of root and crown, *i. e.*, the total weight of root was multiplied by the percentage of sugar. As the crown and tail-end of the root contain a relatively low percentage of sugar the calculated quantities of sugar are somewhat higher than those actually present.

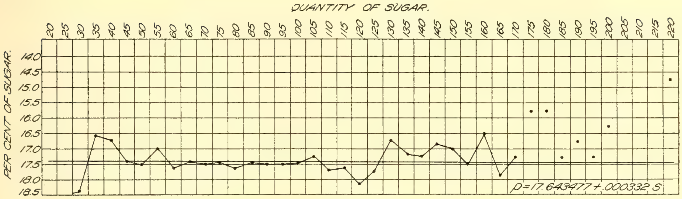

FIG. 7. Relationship between percentage and quantity of sugar per root. (To accompany table III-*b*.)

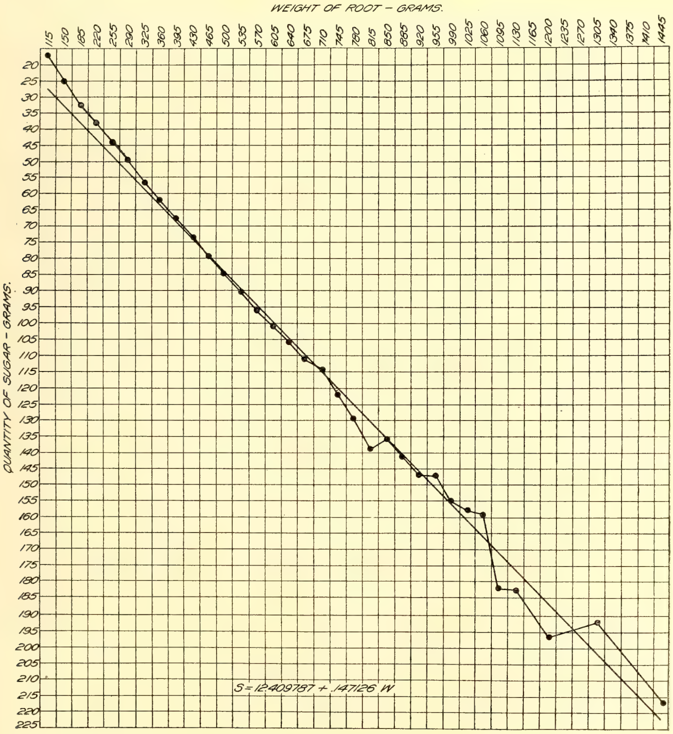

FIG. 8. Relationship between quantity of sugar and weight of root.

TABLES V–XV.

Number of Table	Relationship Between Saccharine Content of Root and	Number of Plants	Weight of Root in Grams, Mean	Quantity of Sugar per Root in Grams		Percentage of Sugar in the Beet	
				Mean	Standard Deviation	Mean	Standard Deviation
V	Shape of root						
	pyriform	1,920	443	78.28± .35	23.16± .25	17.67±.02	1.58±.02
	conical	1,348	452	80.18± .44	24.00± .31	17.75±.03	1.59±.02
	napiform	355	458	80.70± .85	23.93± .60	17.63±.06	1.54±.04
	fusiform	77	510	88.24±2.07	26.96±1.46	17.30±.12	1.56±.08
	cylindrical	84	590	99.88±1.92	26.19±1.36	16.93±.14	1.91±.10
VI	Shape of crown						
	flat..........	961	494	87.17± .55	25.73± .39	17.63±.04	1.63±.03
	rounded	2,579	438	77.63± .30	22.83± .21	17.74±.02	1.56±.01
	conical	244	433	74.83± .94	21.92± .66	17.30±.06	1.74±.05
VII	Character of root furrows						
	direction						
	intermediate	1,462	448	79.36± .40	22.76± .28	17.70±.03	1.56±.02
	vertical	1,874	454	80.15± .38	24.76± .27	17.67±.02	1.61±.02
	spiral	448	455	80.54± .76	24.09± .54	17.70±.05	1.55±.03
	depth						
	medium	1,321	442	78.36± .43	23.24± .30	17.71±.03	1.57±.02
	shallow	1,141	445	78.10± .48	24.45± .34	17.55±.03	1.64±.02
	deep	1,322	468	82.91± .44	23.87± .31	17.72±.03	1.53±.02
VIII	Growing habit of foliage						
	erect	614	468	81.33± .67	24.94± .49	17.37±.04	1.61±.03
	semi-erect	2,852	445	78.98± .29	23.65± .21	17.74±.02	1.59±.01
	flat	318	482	85.03± .89	23.72± .63	17.65±.04	1.00±.03
IX	Color of foliage						
	light green	60	455	79.33±2.28	26.21±1.61	17.44±.15	1.75±.10
	medium	53	440	77.17±1.64	17.81±1.16	17.54±.14	1.53±.10
	dark	3,671	452	79.92± .26	23.97± .18	17.68±.02	1.58±.01
X	Leaf dimension						
	length						
	short	496	411	72.78± .62	20.77± .44	17.69±.05	1.57±.03
	medium	2,695	450	79.60± .30	23.51± .21	17.70±.02	1.55±.01
	long	593	497	87.05± .72	26.24± .51	17.50±.05	1.70±.03
	breadth						
	narrow	105	455	80.52±1.41	21.48± .99	17.70±.12	1.77±.08
	medium	3,599	449	79.30± .26	23.63± .18	17.68±.02	1.57±.01
	wide	80	605	104.75±2.28	30.33±1.61	17.31±.13	1.70±.09
XI	Character of leaf surface						
	smooth	3,500	448	79.70± .27	23.85± .19	17.79±.02	1.58±.01
	wrinkled	284	477	82.04± .99	24.83± .70	17.21±.07	1.72±.05

TABLES V–XV.—*Continued*

Number of Table	Relationship Between Saccharine Content of Root and	Number of Plants	Weight of Root in Grams. Mean	Quantity of Sugar per Root in Grams		Percentage of Sugar in the Beet	
				Mean	Standard Deviation	Mean	Standard Deviation
XII	Leaf texture						
	fine	344	461	80.84± .84	23.34± .60	17.53±.06	1.74±.04
	medium	2,521	453	79.99± .32	24.17± .22	17.65±.02	1.59±.02
	coarse	919	445	79.18± .52	23.49± .36	17.79±.03	1.48±.03
XIII	Character of leaf margin						
	undulate	882	449	79.68± .53	23.67± .38	17.73±.03	1.57±.03
	sinuate	803	430	75.72± .54	23.03± .38	17.60±.04	1.59±.03
	curly	2,099	461	81.54± .35	24.19± .25	17.69±.02	1.58±.02
XIV	Petiole dimension						
	length						
	short	357	439	77.55± .76	21.43± .54	17.67±.05	1.46±.04
	medium	1,297	445	79.13± .43	23.13± .30	17.80±.03	1.56±.02
	long	339	459	80.40± .85	23.44± .60	17.50±.06	1.70±.04
	breadth						
	narrow	998	420	74.02± .46	21.60± .32	17.61±.03	1.60±.02
	medium	2,349	452	80.20± .32	23.18± .22	17.75±.02	1.58±.02
	wide	437	525	91.50± .90	28.17± .64	17.43±.05	1.70±.04
XV	Depth of petiole groove						
	shallow	1,159	419	74.12± .46	23.36± .32	17.67±.03	1.61±.02
	medium	1,282	444	78.51± .43	23.04± .30	17.70±.03	1.57±.02
	deep	1,343	479	84.61± .45	24.46± .31	17.66±.03	.571±.02

When root weight is variable however a very different relationship obtains between percentage and quantity of sugar as shown in table III *b* and its accompanying graph (fig. 7).

There is apparently no correlation between percentage and quantity of sugar in beet roots of miscellaneous weights.

The correlation found between the size of the root and the quantity of sugar it contains is shown in table IV.

The coefficient of .92 is very high. Calculating the regression equation and expressing this relationship in the form of a graph (fig. 8) we obtain a striking illustration of the role of size in influencing the quantity of sugar independently from the percentage of sugar.

The shape of a beet root as shown by Plot (4) and others, affects its sugar content. The five most common forms were studied and their relative values recorded in table V. The relative frequency of each type corresponds to its numerical proportion of the number of plants in each group.

As shown by the table, the cylindrical roots were lowest in percentage but highest in quantity of sugar. The conical form had the highest average percentage, but when the probable errors are considered there is no real difference between it and the pyriform and napiform types.

The relative merits of three different types of crown, viz., flat, rounded and conical, were also determined and the results summarized in table VI.

Beets having flat crowns were heaviest and contained a slightly higher percentage of sugar than roots possessing conical crowns, which is contrary to expectation, as the larger roots, as a rule, contain the lower percentage—a difference of 35 grams in weight being equivalent to a difference of about 1-10 of one percent sugar (cf. fig. I). The conical crown, therefore, appears to be a detrimental character as it is correlated with both a low percentage and a small quantity of sugar.

Deep, spiral root-furrows are said to denote contraction or density of the root and hence a high percentage of sugar. In order to determine the value of depth and direction respectively, the data for each character were tabulated separately and summarized in table VII.

There appears to be no correlation between direction of furrows and percentage or quantity of sugar. It is different, however, in regard to depth of furrows. Shallow furrows are apparently another character of relatively low breeding value. Deep furrows, on the contrary, are correlated with a large quantity of sugar, and roots possessing them show no diminution in percentage of sugar when compared with somewhat smaller roots with shallow furrows.

Considerable difference of opinion prevails regarding the most desirable growing habit of beet foliage, *i. e.*, direction assumed by the leaves. Leaves rising above the ground and having an approximately right-angled exposure to the sun's rays are generally preferred, but the flat or rosette type also has its adherents. In table VIII of the present paper, three types of foliage are compared; erect, semi-erect, and flat.

As shown in the table, the flat or rosette type is correlated with heavy weight and high percentage of sugar, while the erect type indicates low percentage. Although the difference in percentage is very small when due regard is given to probable errors and a proper allowance made for difference in root weight, these results tend to confirm those of Vychinski (5), Karmrodt,[5] and Marek,[6] who claim that flat foliage is correlated with a higher percentage of sugar than are leaves of the erect type. The semi-erect type, however, seems to be fully as desirable as the flat.

The relationship of chlorophyll to photosynthesis suggests a possible correlation between color of foliage and sugar content but as an abundance of nitrates usually stimulates growth and imparts a deep green color to the foliage, and beets grown under these highly nutritive conditions are often low in sugar, the significance of color is not even theoretically established. The experimental results obtained are summarized in table IX.

When due consideration is given to probable errors no constant relationship is exhibited between percentage of sugar and color of foliage.

It has been fairly well proven that quantity of leaves[7] and yield of sugar per acre are positively correlated, but the quantity of leaves per beet is difficult to determine at the time of harvest as beet leaves complete their growth in from four to six weeks, then gradually dry up and drop off as new leaves are developing. Therefore, the relationship was determined between sugar content and leaf dimension. The results are presented in table X.

The data show that size of root and quantity of sugar increase with leaf dimension.

As the character of the leaf surface may influence the amount of photosynthetic work done by the plant, two types, namely, smooth and wrinkled, were compared. Their average performance is expressed by the biometrical constants of table XI.

The chief difference between these types lies in their correlation with percentage of sugar. Beets having smooth leaves were the richer, which is directly opposite to the results reported by Vychinski (5), who claims that saccharine richness increases directly as the quantity of wrinkling.

Three groups of beets were made upon the basis of leaf texture and their relative values recorded in table XII. As used in this paper, texture refers chiefly to thickness and pliability, a thin blade easily folded denoting fine texture.

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[5] Cited by Plahn (6).

[6] Cited by Fruwirth (1).

[7] Maercker, cited by Plahn (6).

Contrary to expectation, fine leaf texture was found to be correlated with large roots. Ordinarily this would signify a greater quantity of sugar but a lower percentage, which is hardly substantiated by the present table although the difference in percentage is slightly in excess of three times the probable error of the difference.

Three types of leaf margin were compared; undulate, sinuate, and curly. The curliness was confined to the outer portion of the leaf, at and near the margin. The relative efficiency of the types for producing sugar is shown in table XIII.

The data show no difference in the value of these types. The plants having a sinuated border produced the least sugar, but this was due to the small size of their roots.

From a knowledge of correlations between the various parts of an individual, we should expect to find large petioles correlated with large roots and hence with a large quantity but low percentage of sugar. The relationship of petiole dimension to sugar content is shown in table XIV.

There is apparently no preference in petiole dimension with respect to percentage of sugar when due allowance is made for differences in root weights (cf. fig. 1). The total sugar, however, increases with the size of the petiole, which is especially marked in connection with breadth.

The groove in the upper surface of the leaf stalk was divided into three grades on the basis of depth and a summary, table XV, was made of their relationships to percentage and quantity of sugar in the root.

As both size of root and quantity of sugar vary with the depth of groove in the petiole without any dimunition in percentage of sugar, this character appears to have an important bearing on yield.

Synthetic Types

By aid of the foregoing data, three different types of sugar beets, designated respectively by the letters *A*, *B* and *C*, have been formed by combining in *A* characters correlated with relatively low sugar production and in *B* and *C* characters correlated with both a large quantity and a relatively high percentage of sugar. Type *A* was based entirely upon the character of the crown and root furrows, as the addition of other characters would have eliminated many roots and made the number otherwise available composing this class too small for satisfactory comparison. Types *B* and *C* contain two extra characters, viz., type of leaf surface and depth of petiole groove, which are probably significant, and a number of other neutral characters which as shown in preceding tables have no bearing on sugar production. While types *B* and *C* are opposite to type *A* only in the characters which constitute *A*, they are nevertheless sufficiently distinct to determine whether morphological characters play any part in the relative performance of beet types.

Description of Types

Type *A*

Crowns: conical. Root furrows: shallow

Type *B*

Root:
    Shape: conical (neutral)
    Crown: flat or rounded
    Furrows: deep
Foliage:
    Color: dark green (neutral)
    Habit of growth: semi-erect or flat (neutral)
Leaf:
    Surface: smooth
    Texture: smooth
    Texture: medium or coarse (neutral)
    Relative area: medium (neutral)
Petiole:
    Groove: deep

Type *C* is identical with *B* except in root form, type *C* having pyriform roots.

The data belonging to these types were separated from those of the general population and tabulated under their respective headings. Hence, no breeding was done. Records of beets embodying the different characteristics were merely picked out from the general population. Both the frequency distribution and the relative behavior of the types are shown in the following tables.

Table XVI

Frequency Distribution of Beets Arranged According to Percentage of Sugar in the Beet

Type	13.0	13.5	14.0	14.5	15.0	15.5	16.0	16.5	17.0	17.5	18.0	18.5	19.0	19.5	20.0	20.5	21.0	21.5	22.0	No. of Plants	Mean	Standard Deviation
A	1	1	1	2	3	5	9	12	12	6	11	5	6	4	2					80	17.07±.11	1.48±.08
B				3	1	3	8	6	13	14	11	10	5	6	2	3			1	86	17.63±.11	1.44±.07
C					2	4	3	9	11	11	12	13	7	8	4	1	2		1	88	17.93±.10	1.40±.07

Table XVII

Frequency Distribution of Beets Arranged According to Quantity of Sugar per Root in Grams

Type	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	135	140	145	150	No. of Plants	Mean	Standard Deviation
A .	1	1	1	2	0	5	7	8	7	9	10	7	2	5	5	0	3	1	1	1	1	1	1			1	80	74.68±1.75	23.18±1.24
B .			1	0	0	2	5	5	9	8	5	4	6	5	7	7	1	10	3	1	0	3	0	3	0	1	86	87.26±1.76	24.22±1.25
C..				3	4	2	1	2	5	7	4	9	11	7	7	6	7	3	3	2	3	2					88	85.51±1.57	21.88±1.11

The number of individuals in each group is not large but the distributions are fairly regular. While the types do not differ much in behavior, *B* and *C* exceed *A* by several times the probable error both in percentage and quantity of sugar per root. If wrinkled leaf surface and shallow petiole groove had been included in Type *A* the contrast between it and the other types might have been still greater. It is possible that if certain other characters as number of woodrings, size, shape and number of bundles within the woodring, number of leaf-circles (spiral turns), total number of leaves, and character of veining had been included in the investigation, greater differences would have been exhibited between types.

SUMMARY

A statistical study of 3,784 individual sugar-beet plants grown at Brookings, S. D., was made to determine the correlations which exist between certain morphological characters of the plant and the percentage and quantity of sugar in its root. Data collected at Fairfield, Washington, were also used in studying the relationship between weight of root and percentage of sugar, but no data from different years nor from beets grown in different fields were grouped for this study. Hence each biometrical constant was determined from a single year's crop produced under fairly uniform field conditions. The results obtained from this material were as follows:

1. The coefficients of correlation found between weight of root and percentage of sugar were —.253, —.258, —.254, —.257, and —.499; between weight of root and quantity of sugar, .920.

2. The correlation between percentage and quantity of sugar in roots of 35 grams range in weight was nearly perfect, viz., .93, .96, and .99 but in roots of miscellaneous sizes no correlation was apparent between percentage of sugar and quantity of sugar per root.

3. No correlation was found between the sugar content of the root and type of leaf margin or color of foliage.

4. Shallow groove of petiole, conical crown, and shallow root furrows showed a very small amount of correlation with a relatively low quantity and relatively low percentage of sugar; flat crown, deep root furrows, deep groove of petiole, smooth leaf surface, flat foliage and coarse leaf texture also showed slight evidence of correlation with a large quantity and relatively high percentage of sugar. These correlations were so small, however, as to cast some doubt upon their permanence and practical significance.

5. Contrasting types of sugar beets were formed by making separate combinations of characters slightly correlated with low performance and characters slightly correlated with high performance. The records of beets embodying these characters were picked out from those of the general population and placed in their respective groups. The behavior of the types as shown by their mean values corresponded to the class of characters composing them but the differences were very small.

In conclusion the writer wishes to acknowledge his indebtedness to Dr. J. Arthur Harris for many helpful suggestions.

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MUTATION IN MATTHIOLA ANNUA, A "MENDELIZING" SPECIES[1]

Howard B. Frost

Interest in the subject of mutation seems to be growing, and with good reason. Varied as may be the processes concerned in the production of mutant forms, evidence is accumulating to show that sometimes, at least, mutation involves fundamental chemical or structural changes in the germ-plasm. It is obviously of great importance in practical breeding, and we cannot yet safely deny to it a significant rôle in evolution.

In view of the peculiarities in the general genetic behavior of Oenothera, it has become a matter of special importance to study mutation in plant forms that are known to exhibit typical Mendelian phenomena. Two general views of heredity in Oenothera appear still to be possible; first, the one recently expressed by Shull (1913), that regular segregation and recombination of genetic factors are absent; second, that the irregularity is secondary, and due to complicating influences, especially to selective sterility. Perhaps the genus possesses peculiarities of chromosome structure and behavior which will be found to justify a view practically intermediate between the two just stated.

It now seems very probable, whatever the fundamental reason, that other genera are to be classed with Oenothera as possessing certain genetic peculiarities. In Citrus, for instance, we find a similar tendency toward apparent mutation, which is very strikingly manifested by somatic tissues. Here, also, sterility seems to be common, as with the Washington navel orange, where it is complete in the anthers, and with the Satsuma mandarin, where very few of the pollen-grains are capable of germination. Probably the potato (or the genus Solanum?), with its apparent somatic mutation, its doubtfully regular genetic ratios, and its very general pollen-sterility, also belongs to this group.

If Oenothera is thus typical of a group of genera, we may expect to find this group characterized by a peculiar prevalence of hybridity. Apparently this hybridity is not due to unusual opportunities for interpollination of species in their past history; it is rather to be ascribed to an exceptional degree of fertility between decidedly unlike forms, perhaps combined with an unusual tendency to produce widely divergent forms by mutation. Frequent partial sterility of the resulting hybrids, sometimes producing *permanently heterozygous* forms, seems also to be a factor (de Vries, 1913).

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It has been urged that all the Oenothera mutations should be charged to hybridization. There is obviously a remarkable prevalence of hybridity in the genus, but we may well consider whether this hybridity may not be, in large part, a *result* rather than a *cause* of mutation.

However this may be, Belling (1914) seems to have shown that the artificial crossing of two "good species" may lead to the opposition (pairing), at the reduction division in the hybrid, of non-homologous genes, with the result that some germ-cells are not viable. That is, certain germ-cells may receive incompatible materials, or lack essential ones. If, then, a genus combines high mutability with wide limits of interfertility of forms, the resulting germ-plasms may be very unsymmetrical in the structure of their pairs of chromosomes, often producing non-viable gametes, and may therefore be apparently "non-Mendelian" in transmission of characters. It seems very significant, in this connection, that the various "species" of Oenothera and of Citrus are so generally interfertile.

Von Tschermak (1912) has made a specially extensive experimental test of the factor-hypothesis of heredity, and concludes that his evidence is very favorable to this hypothesis. One of the three plants which he used is the ten-weeks stock, *Matthiola annua* or *M. incana* var. *annua*, which Miss Saunders (1911) also has found to give typical Mendelian results for flower-color and pubescence. Mutations similar to those of Oenothera, occurring in such material, acquire a special significance.

In Annual Report 8 of the American Breeders' Association, I (Frost, 1912) reported the discovery of a series of such mutations in stocks, discussing especially a few-noded type of apparently regular heredity. Six or seven of these types have reproduced themselves in progeny-tests, some through several generations, but only the early (few-noded) type has been found to be evidently homozygous in any case, even in the later generations. With all the other types, a large part of the progeny, usually more than one half, always belong to the type ("Snowflake") from which the mutant types were originally secured.

Possible explanations of this peculiar transmission of the mutant types will be discussed in a paper now in preparation, and cannot be adequately considered here; lower viability of the mutant types, and linkage with the factor for the less viable single-flowering type[2] are obviously concerned in some cases. The point to be emphasized here is that this peculiar behavior occurs in a species which, as to certain other factors, is typically "Mendelian."

A brief characterization of eight of the mutant types, including all that have been proved to be hereditary, will be given. It should be noted first that the foundation stock consists of one variety only, a very uniform white-flowered glabrous "double-throwing" form, known as "Snowflake." As with other double-producing stocks, all the single-flowering plants are heterozygous for "doubleness," producing about fifty percent[3] of double progeny, while the doubles are totally sterile. The fact that the doubles form about one half of each generation, rather than one fourth, is due to the fact that all functional pollen is doubleness-carrying, or lacks some factor necessary to single (or normal) flowers.

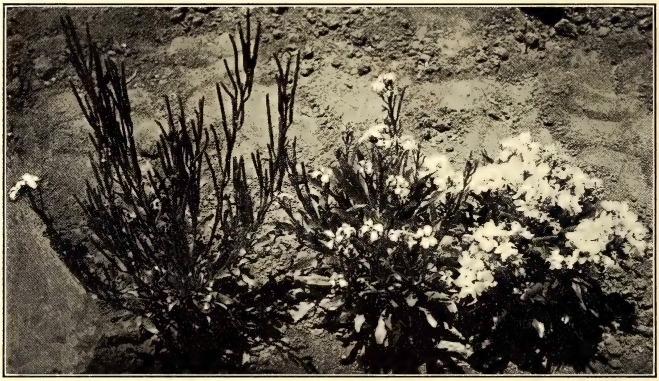
The early type, which was described in Annual Report 8 of the American Breeders' Association, differs from Snowflake (figs. 1, 2 and 3) mainly or entirely in size-characters and earliness of flowering. The original early mutant was heterozygous. Twenty of its progeny have been tested; 4 of these were probably pure early, and 7 were obviously pure Snowflake. Presumably the mutation occurred in one germ-cell, which then united in fertilization with a normal Snowflake germ-cell. In the absence of complications, such a mutant would be expected to reproduce its type in about 75 percent of its progeny—as actually occurred with this type.

In striking contrast to this case, all the other mutant types identified are marked by peculiarities of leaf-form and of general habit, and no individual yet tested has proved homozygous.

[2] The single-flowering plants appear somewhat less vigorous, and less resistant to some unfavorable conditions, than the double-flowering ones.

[3] There is the usual slight excess of doubles; 7,310 progeny of Snowflake parents included 53.037 percent of doubles.

One of the most interesting of all the mutant types is a *gigas*-type, designated "large-leaved" (fig. 1). This has leaves longer and thicker than those of the Snowflake type, with stout stem and capsules, and is late in blooming. About half of its progeny reproduce its type, while nearly all the rest are Snowflake. Evidence is not yet at hand to explain the deviation from the expected seventy-five percent of large-leaved progeny, though there are several evident possibilities.



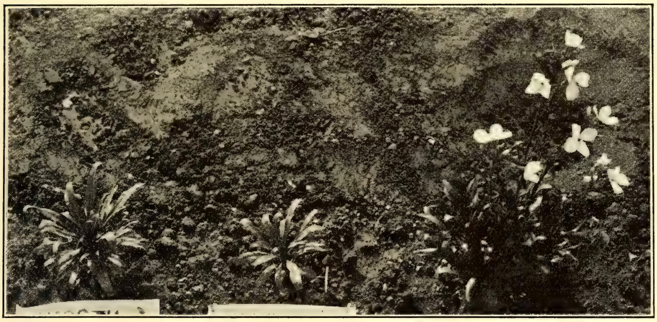
THE LARGE-LEAVED AND NARROW-DARK-LEAVED TYPES

FIG. 1. Matthiola plants at Riverside, California, in 1914–15. Progeny of a Large-leaved parent. Types, from left to right: Snowflake, Large-leaved, Narrow-dark-leaved. Observe especially the lateness of the Large-leaved plant (it should be noted that this type has seemed to seed quite as freely as Snowflake when grown to maturity under favorable temperature-conditions).

The smooth-leaved type (fig. 2) was named from the lack of buckling between the main veins in the leaves of young seedlings grown in the greenhouse. In field cultures, with injuriously high temperature and at times deficient moisture, the leaves become dotted with small brown spots. Flowering is late, and the mature stems are brittle. Probably the fibrovascular system is in some way defective.

The crenate-leaved type (fig. 3) has more crenation or serration of the leaf-margins than has Snowflake, under some conditions much more; the plants are small and slender, and under unfavorable conditions may be extremely dwarfed.

The narrow-leaved type is characterized by rather rigid narrow leaves, flat or concave upward, ascending rather than horizontal; the sepals and petals are conspicuously narrow. The narrow-dark-leaved type (fig. 1) is in some respects more like Snowflake; its leaves tend to be small, very dark green, and smoothly convex upward, while the sepals are probably normal. Two variant parents, grown before this last type was recognized, have given it among their progeny; one of these, however, apparently gave four to six other definite types, including Snowflake as usual.



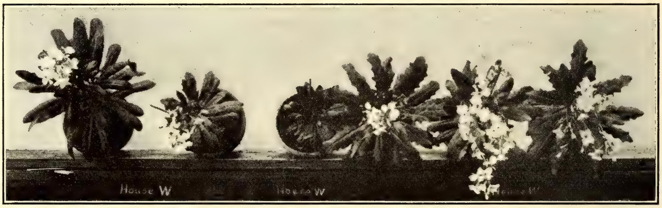
THE SMOOTH-LEAVED TYPE

FIG. 2. Matthiola plants at Riverside, California, in 1913–14. Progeny of a Smooth-leaved parent. Types, from left to right: Smooth-leaved, Smooth-leaved, Snowflake. Note the lateness of the Smooth-leaved plants, and the punctate appearance of some of the leaves (due to small dead spots.)

The slender type, under favorable conditions, is much like Snowflake, but with somewhat lengthened internodes, petioles, and pedicels. Under some unfavorable conditions it may be much dwarfed, although it seems to endure high temperatures better than Snowflake. Two grades of this type have been found; the extreme form, in one small test, gave the larger proportion of slender progeny, but still only nine plants out of sixteen. In this last case the plants were all doubles, and elsewhere there is, apparently, linkage of a specially puzzling sort with the single-double factor-pair.

The last five types described have all given considerably less than 50 percent of mutant-type progeny.

The small-smooth-leaved type is the smallest and weakest of all that have been named. Two individuals, both singles, have flowered, but no seed was produced. Several other forms have been named, but have failed to give seed; it is very probable that at least two or three of these are definite genetic types, distinct from any discussed above. In fact, it is not improbable that as many as fifteen distinct genetic types have been observed, some perhaps only once among about 8,000 plants.



The Crenate-leaved Type

Fig. 3. Matthiola plants grown in greenhouses at Ithaca, N. Y. Progeny of Snowflake parents. Types: one Snowflake plant at the left in each row, the rest Crenate-leaved mutants. Note the crenation or serration of the leaf-margins, which here is nearly absent with Snowflake but usually very marked with Crenate-leaved.

Many Snowflake parents have given mutant progeny. The number of apparent mutants, under favorable conditions for germination and growth, was about four or five percent of the whole number of progeny. The commoner forms appeared many times, as progeny of many parents; it seems probable that any Snowflake parent, self-pollinated, can give rise to any of the mutant types studied. It is, however, probable that plants of some mutant types produce other mutant types more frequently than does Snowflake.

The mutant individuals are obviously not extracted pure recessives, but heterozygous dominants. Since they have occurred many times in cultures from selfed parents, they are not due to combination of complementary factors by cross-fertilization. They would seem, then, to be due to definite changes in the germ-plasm, changes distinct from those shiftings which produce ordinary Mendelian phenomena. Whether past hybridization has made these mutative changes possible, is an open question; we know that new types may be formed by hybridization, but the known methods of such type-formation seem to be out of the question here.

Finally, it should be noted that in most of these cases the mutative change has far-reaching effects, modifying various characteristics of the plant. Notwithstanding this fact, the factor for the new type is regularly inherited as a unit, and sometimes shows linkage with another factor-pair, so we may suppose that the essential change is limited, in some cases at least, to a portion of one chromosome.

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THE GROWTH OF FOREST TREE ROOTS

W. B. McDougall

It has become a commonly accepted view that the growth of tree roots takes place in spring and in autumn, and that there is a period of rest in mid-summer as well as in winter. The workers who have published on this subject, however, do not all agree as to the time of year of the growth and rest periods, and no attempt seems to have been made by any of them to determine to what factor of the environment, if any, a summer rest period might be due.

Resa (1), who was the first to work intensively on the subject, came to the conclusion that deciduous-leaved trees produce their new roots in the autumn, while coniferous trees produce them in both autumn and spring, the exact time depending upon the weather. In the deciduous-leaved trees he found some root growth in the spring but not the formation of new roots. He also stated that the autumn growth period persisted, to a certain extent, throughout the winter, the period of root growth not corresponding at all with that of aerial growth. Wieler (2), on the other hand, found root growth only in the spring; but Petersen (3), Hämmerle (4), and Büsgen (5), all agreed with Resa that growth occurs in autumn as well.

Methods

The work on which the present paper is based was carried on in the "Forestry," a small artificial wood-lot at the University of Illinois. Two methods, or rather two modifications of one method, were used for making observations on the same roots at intervals throughout the growing season: (I) The horizontal glass-plate method. This method is similar to that used previously by the author (6) in making direct observations on developing mycorrhizas. The leaf mold and humus were scraped away to a depth of about two inches or until some healthy roots were exposed. These roots were then covered with a square of window glass, one foot square. Over this was placed a square of felt roofing and the whole was then covered with soil. The roofing kept the glass fairly clean but, nevertheless, it was usually necessary to remove the glass when an observation was to be made. (II) The vertical glass-plate method. In order to make observations on roots located somewhat deeper than was possible by using horizontal plates, holes were dug in the earth two and one-half feet wide by five feet long and two feet deep. A glass plate, one foot square, was then placed against the soil and roots at one end of the hole in such a way as to exclude any extensive air spaces between the glass and the soil, and held in place by means of long pins made of number nine wire. A piece of felt roofing was placed against the glass to exclude light and was held in place by board props. The hole was then covered with a board cover hinged to two stakes at one side and locked with a padlock to a third stake at the opposite side.

Observations were made by both of the above methods on two individuals of each of the following four species of trees: *Acer saccharinum* L., *Tilia americana* L., *Carya laciniosa* (Michx f.) Loud., and *Quercus alba* L. The holes were dug and the glass put in place during December, 1913. They were first visited for observation on January 5, 1914, but regular observations did not begin until the twenty-eighth of April of that year. The work was continued until September 2, 1915. During the warmer parts of the year observations were made weekly except on two occasions, one in August, 1914, and one in July, 1915, when I was out of town for periods of two weeks. During the winter, observations were made usually only when the soil was not frozen. During the warmer part of the summer it was found that observations could not be made during the middle of the day without the roots being injured or killed by exposure to the warm dry air, especially on clear days. For that reason observations were regularly made just at or just after sunrise. In spite of this precaution many rootlets had their growth checked or even stopped permanently by exposure while observations were being made.

At each observation a chart of the observation field was made and the position of each fresh-looking rootlet was indicated thereon. At the same time the length of each rootlet was measured and recorded. Growth was recorded only when an increase in length could be detected in a week's time by measuring with an ordinary millimeter rule.

RESULTS IN 1914

Acer saccharinum: Growth had already begun at the time of the observation on April 28. At that time the leaves were about half developed. The trees were through blooming and the fruit, while still green, was nearly mature. The branches were growing rapidly.

The roots grew rapidly and many new rootlets were formed during May and June. June was a very dry month and July was still drier, so that by the middle of July we were in the midst of a pronounced drought. During the fore part of July the rate of root growth gradually lowered until by July 14 growth was almost at a minimum. This very slow rate of growth continued during the rest of July, often not more than a millimeter of elongation being detected in a week's time, and that only in a few rootlets. On August 6 growth had apparently entirely ceased and no further elongation could be detected during the remainder of August. The soil during this month was extremely dry. On August 28, however, it rained all day, and on September 8 growth had been resumed. During the remainder of September the roots continued to grow slowly. During the greater part of October no elongation could be detected, but on October 31 some growth was detected, and this continued until the end of November. No growth was detected after December 1. The month of December was very cold, being several degrees below zero during part of the time.

Tilia americana: The roots had already started growing on April 28. They grew rapidly during May and June, but after the first of July the rate of growth began to lower and by July 28 all elongation had ceased. On September 8 some growth was again detected, and the roots continued to grow slowly until November 24 after which no elongation was recorded.

Carya laciniosa: As in the above cases growth had already started on April 28, and continued through May and June. In July the rate of growth diminished and on July 28 it had ceased under both of the horizontal plates and under one of the vertical plates. Toward the bottom of the other vertical plate, however, the roots were still growing and they continued to grow unceasingly throughout the remainder of the summer. At no time, when an observation was made, was it found impossible to detect some elongation of these roots until after November 24. The roots under the other three plates had started growing again on September 8 and continued to grow, though rather slowly, until November 24, after which no growth was recorded for any of the hickory roots.

Quercus alba: The first observations on the oak roots were made on May 5. At that time growth had already started. The roots grew rapidly until after the first of July and then slowly until July 28. On July 28 growth was recorded only in a single rootlet near the base of one of the vertical plates. No further growth was detected until September 8 when it was noted that elongation had taken place in several roots under the vertical plates. These roots continued to grow during the rest of the season, but the first record of growth for roots under the horizontal plates was obtained on October 15. After this date there was growth under all of the plates until November 24, after which no further elongation was detected.

RESULTS IN 1915

The results in 1915 can be stated rather briefly. No observations were made on *Tilia americana*, during this year, for the reason that three of the preparations had been destroyed and a fungus had vegetated so freely under the fourth plate that it was considered useless to attempt further observation. For similar reasons observations were made on only one tree of *Acer saccharinum* instead of two.

The first observations for the year were made on March 23. At that time the flower buds of the soft maples were opening but no root growth was noted on either the maples, hickories or oaks. The first positive evidence of growth was obtained on April 5 on maple and hickory, and the first growth detected on the oaks was recorded on April 13. Warm spring weather began on April 5 and warm rains occurred on April 9 and 10. After April 13 the roots grew continuously as long as observations on them continued. At no time during the season when observations were made was it impossible to detect growth in the roots of all three species. The last observations were made on September 2 when the roots were still growing well. There was an abundance of rain throughout the season; there was no period of drought.

Tables I and II show in abbreviated form the data upon which the foregoing summary is based. It will be noted that the glass plates are designated by means of a numeral and a letter *A* or *B*. In every case the numeral is the number given to the individual tree, while *A* indicates a horizontal plate and *B* a vertical plate. A + in the tables indicates that some elongation was observed in at least one root, while a — indicates that no growth was detected.

TABLE I

1914 Results

Date	*Acer saccharinum* Plates				*Tilia americana* Plates				*Carya laciniosa* Plates				*Quercus alba* Plates			
	1*A*	1*B*	2*A*	2*B*	3*A*	3*B*	4*A*	4*B*	5*A*	5*B*	6*A*	6*B*	7*A*	7*B*	8*A*	8*B*
Jan. 5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Apr. 28	+	+	+	—	—	+	—	+	—	+	+	+				
May 5	+	+	+	—	+	+	+	+	+	+	+	+		+	+	+
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+
26	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+
June 4	—	+	+	—	+	+	+	+	—	+	+	+	+	+	+	+
11	+	+	+	+	+	—	+	+	—	+	+	+	+	+	+	+
20	+	+	+	+	+	—	—	—	—	+	+	+	+	+	+	+
30	—	—	+	+	+	—	—	—	—	—	+	+	+	+	+	+
July 7	+	—	—	—	+	—	—	+	—	—	+	+	—	+	+	+
14	+	?	—	+	+	—	—	+	—	—	+	+	—	+	—	+
21	—	+	—	+	+	—	—	+	—	—	+	+	—	—	—	+
28	—	+	—	+	—	—	—	—	—	—	—	+	—	—	—	+
Aug. 6	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—
13	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—
29	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—
Sept. 8	+	+	+	—	+	—	—	+	+	+	+	+	—	+	—	+
16	+	+	+	+	+	+	—	+	+	+	+	+	—	+	—	+
19	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	+
29	—	—	+	+	+	+	—	—	+	+	+	+	—	+	—	+
Oct. 7	—	—	+	+	+	—	+	—	+	+	+	+	—	+	—	+
15	—	—	—	—	+	—	+	+	—	+	+	+	+	+	+	+
20	—	—	—	—	+	—	—	+	+	+	—	+	+	+	+	+
31	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nov. 7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dec. 1	—	+	+	+		—	—	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

DISCUSSION

The cause of periodicity in plants has been the subject of much discussion. Schimper (7) concluded from his own observations, and the observations of other workers in tropical regions, that plants in general of necessity show periodicity, and that this is due to internal causes. Klebs (8), on the other hand, brought forward considerable evidence against Schimper's interpretation and denied the existence

TABLE II

1915 Results

Date	*Acer saccharinum* Plates		*Carya laciniosa* Plates				*Quercus alba* Plates			
	2A	*2B*	*5A*	*5B*	*6A*	*6B*	*7A*	*7B*	*8A*	*8B*
Mar. 23.....	−	−	−	−	−	−	−	−	−	−
Apr. 5.....	+	+	+	−	−	+	−	−	−	−
13.....	+	+	+	+	−	+	+	+	−	+
20.....	−	+	+	+	+	+	+	+	−	+
26.....	+	+	+	+	+	+	+	+	+	+
May 8.....	−	+	−	+	+	+	+	+	+	+
17.....	+	+	+	+	+	+	+	+	+	+
25.....	+	+	+	+	+	+	+	+	+	+
June 3.....	+	+	−	+	+	+	+	+	+	+
12.....	+	−	+	+	+	+	+	+	+	+
12.....	+	+	+	+	−	+	+	+	+	+
26.....	+	+	+	+	+	+	+	+	+	+
July 5.....	+	+	+	+	+	+	+	+	+	+
20.....	−	+	+	+	+	+	+	+	+	+
27.....	−	+	+	+	+	+	+	+	+	+
Aug. 3.....	−	+	+	+	+	+	+	+	+	+
10.....	+	+	+	+	?	+	+	+	+	+
18.....	+	+	+	+	−	−	+	+	+	+
25.....	+	+	+	+	+?	+	+	+	+	+
Sept. 2.....	−	+	+	+	−	+	+	+	+	+

of periodicity independent of external factors. If the cause of a periodic phenomenon is internal, then, of course, there can be nothing gained by searching for a cause in the external environment. On the other hand, if the cause is external, then it is surely capable of being discovered, and, if an adequate cause is found in the external environment, then there is certainly no need of presupposing any mysterious internal factor.

Much evidence might be cited in support of the view that periodicity is due to external factors. It is well known that the resting period in various plants can be shortened by etherization or other means, and that many plants which, under natural conditions, have well-marked resting periods, may by artificial means be made to grow continuously. Appleman (9) has shown that the rest period of potato tubers is not of internal origin, but is dependent on external factors, the most important of which is the oxygen supply. According to Brown (10) the termination of the latent period in *Pinus strobus* is dependent upon three external factors; moisture, temperature and available reserve foods.

The results recorded in the present paper show conclusively that the resting period of the roots studied are not fixed and hereditary, since, in 1914, although most of the roots under observation had a summer rest, some of the hickory roots did not have; and in 1915 there was no summer rest period in any of the roots studied, unless it occurred after September 1, which would be most unlikely. Therefore an external cause of the rest period, when it does occur, must be looked for. The two most important factors in the physical environment, that vary with the seasons, are temperature and moisture. A little study of the results given shows that the lowering of either the temperature or the moisture content of the soil retards or stops root growth. In 1914 there was very little rainfall from early spring until the end of August. The soil thus became progressively drier and reached a minimum of water content toward the end of August. The rate of root-growth also gradually decreased and ceased entirely in most cases some time in July, to begin again only after the heavy rains of August 28. In other words, the summer period of rest was only during the period of drought. In 1915 there was no period of drought and, naturally, no rest period. The hickory roots which did not have a rest period during the summer of 1914 were some of the most deeply located roots upon which observations were made, and, naturally, the soil was not so thoroughly dry at that depth as nearer the surface. It is probable that observations on still deeper roots would show all roots located where adequate moisture was available growing throughout the period of drought. Brown (10) found that in the aerial parts of the pine growth is retarded first in the upper portions of the tree and may continue for several weeks longer below. It is very probable that in the subterranean parts a similar difference between the upper and lower portions would be observed on the approach of a critical season.

It seems reasonable to conclude, then, that the summer rest period, when it occurs, is due not to any inherent tendency toward periodicity but to a lowering of the water supply. As to the winter rest period, the results show a close relation to temperature. But temperature to a certain extent controls the water supply, since a lowering of the temperature renders absorption increasingly difficult and thus reduces the amount of physiological water. In this case, therefore, the rest period is due indirectly to temperature but more directly to a decrease in the available water supply.

MYCORRHIZAL RELATIONS

In a previous paper (6) I stated that ectotrophic mycorrhizas are formed in late summer and autumn. In the light of the present investigation it would seem that the time of formation would vary more or less with the season. Two conditions are necessary for the formation of mycorrhizas; the roots must be growing and the proper fungus must be present and in an active and receptive condition. In the season of 1912, when the above mentioned work on mycorrhizas was done, the spring was wet enough for root growth but the early summer was very dry, while from the latter part of July on there was again plenty of moisture. Since no mycorrhizas were formed in the spring it may be supposed that the second condition mentioned above, the presence of a suitable fungus, was not fulfilled. So little is at present known concerning the ecology of the mushrooms that cause mycorrhizas that it is perhaps idle to speculate on their condition and activities in the spring of the year, but it is known that mycorrhizas are produced largely by the later fruiting mushrooms rather than by the spring forms. Since the fruit bodies are usually produced soon after the fungi have become attached to roots, it is reasonable to suppose that they are not in a condition for mycorrhiza formation earlier in the season.

Three of the species of trees used in the present investigation produce ectotrophic mycorrhizas, the oak (*Quercus*), hickory (*Carya*) and linden (*Tilia*). The mycorrhizas of the oak are due to *Russula foetentula* Pk., those of the linden to *Scleroderma vulgare*, Fr., and those of the hickory probably to *Laccaria ochropurpurea* (Berk) Pk., though this last has not been definitely proven. In all of these cases no mycorrhizas were formed in the spring, but after the first of July mycorrhizas were formed whenever the roots were growing well.

CONCLUSIONS

1. The root growth of forest trees begins as early in spring as the soil becomes warm enough for absorption and ceases in autumn when the soil becomes too cold.

2. There is not necessarily a summer resting period.

3. When there is a summer resting period it is due to a decrease in the water supply and not to any inherent tendency toward periodicity.

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THE ANGULAR MICROMETER AND ITS USE IN DELICATE AND ACCURATE MICROSCOPIC MEASUREMENTS

HOWARD E. PULLING

In the formulation of a cytological problem that necessitated the accurate measurement of extremely small bodies it became evident that the commercial ocular micrometers were neither sensitive nor accurate to the required degree. Although it is generally recognized that any instrument whose precision depends upon the accuracy and lack of variation in the pitch of a screw is subject to large errors of construction and to further errors resulting from wear upon the screw and its bearings, it is often believed that the best filar micrometers are sufficiently accurate for all general needs of the microscopist. That this conclusion is not warranted unless each instrument is systematically investigated for error and the necessary corrections made has been shown by Gray,[1] who points out further that satisfactory instruments may easily be rendered worthless by dust particles that grind the screws unevenly and that in all cases instruments must be frequently investigated for error.

That such general observations as these are of interest not only to workers who demand the very highest degree of accuracy from micrometers but that they affect *all* microscopists who use such instruments is evidenced by the following example drawn from my own experience. A filar micrometer, one of the best types obtainable, was used to measure the same fixed distance over different portions of the screw. Through the exercise of great care in the selection of the object and in the manipulation of the instrument the readings made on any one position of the object did not vary from the mean of the readings for that position by more than 1/500 of a drum revolution. Notwithstanding this constancy at each position the variations in the readings at different portions of the screw were as large as 17.5 percent of the smaller number and differences of from 5 percent to 10 percent were common. Since no attempt was made to search for errors of great magnitude and since the object measured was the most favorable that could be found (the distance between two points on an irregular ink spot, moved with a rectangular mechanical stage) it is hardly to be doubted that such an instrument cannot be relied upon without investigating it for errors and constructing an error curve, or table, for the full extent of the screw.

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[1] Gray, Arthur W., Micrometer Microscopes. Scientific paper of the Bureau of Standards No. 215 (reprint of Bulletin of the Bureau of Standards, 10: 375–390. November 5, 1913). Washington, D. C., 1914.

Since in designing the angular micrometer each member of the filar micrometer was separately considered with reference to its liability to structural errors and to its influence upon the observational and personal errors of the operator, a brief review of the defects of the latter instrument will curtail the explanation otherwise necessary.

For accurate work, or even for general use with low and medium magnifications, the drum graduations are inadequate and an accessory scale is required. An instrument of precision should be accurately graduated beyond any demands which may be made upon it.

Because the moving parallel lines extend across the field of view at right angles to their direction of movement, the line connecting the two points whose spatial separation is to be measured must be accurately normal to the moving line. Experiments have shown that the line connecting these two points may often be inclined ten or fifteen degrees from the normal and the inclination be undetected unless the rotation was performed with the visual knowledge of the observer. No definite point exists that may be used as a datum point because the ends of the parallel lines are rounded and because the thickness of the cross lines and the sharp angle at which they meet prevent the operator from surely placing the point of their intersection directly over any particular point on the object-image. Averages of several observations are valueless because when one datum point is used all the errors tend to have the same sign and hence do not offset each other and because no two datum points can be selected which have their errors in exactly opposite directions.

Perhaps it is in part such errors as these that led Farmer and Digby[2] to adopt the method of drawing the object with a camera lucida and measuring the figure drawn. This method is open to many objections; tediousness and difficulty of operation would prevent the majority of workers from adopting it.

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[2] Farmer, J. B., and L. Digby. On dimensions of chromosomes considered in relation to phylogeny. Phil. Trans. London, *B*, **205**: 1–25. 1914.

The angular micrometer was designed to eliminate or greatly reduce the errors inherent in the instruments and methods referred to above. The mechanism for magnifying the movement of the pointer-line is rigid and of a nature to reduce mechanical errors of construction to a minimum. These errors are either negligible or such as to admit of ready detection and calculation. The parts subject to wear are only those whose abrasion with ordinary use will not affect the accuracy of the instrument. The pointer line moves so that the datum point selected can always be easily identified and the graduations are accurate beyond the severest demands.

The principle upon which the micrometer is based is the trigonometrical calculation of the chord of a circle. The equation for this calculation is $x = 2r \cdot \sin(\theta/2)$, in which r represents the radius of the circle and θ the angle subtended by the chord, x, whose length is to be calculated. The apparatus by which θ is determined for the chord in question (the distance between two points on a microscopic object) is shown in figure 1. In *A* the micrometer is shown from above, in place on the microscope; in *B*, from the side. The instrument consists of three essential parts, *a*, *b*, and *c*. The plane of the arm *a* is perpendicular to the axis of the microscope. At the outer edge of this arm is a double vernier, *d*, whose center is on the axis of the microscope. Rigidity is secured by the brace, *g*, and the wide collar, *k*, which is split lengthwise and secured by two sets of screws attached similarly to those for the arm *b* (*h–h*, figure 2, *A*). This arm, *b*, is attached by a split collar, secured with screws, *h–h*, to the ocular of the microscope and bears at its outer edge a protractor, *f*, which is graduated to half-degrees and may be read to minutes of arc with the aid of the vernier. In the instrument which has been constructed the radius of the protractor circle is 17.5 cm., so that readings may be readily made with the naked eye. A glass plate, *c*, is placed in the ocular on the interior focal plane and bears a short photographed line which is tangent to some one of the imaginary circles whose centers are on the axis of the microscope. The point at the tip of this line moves, as the ocular is turned in its sleeve, around the circumference of a circle whose center is on a line which passes through the centers of the protractor and vernier and is perpendicular to their planes.

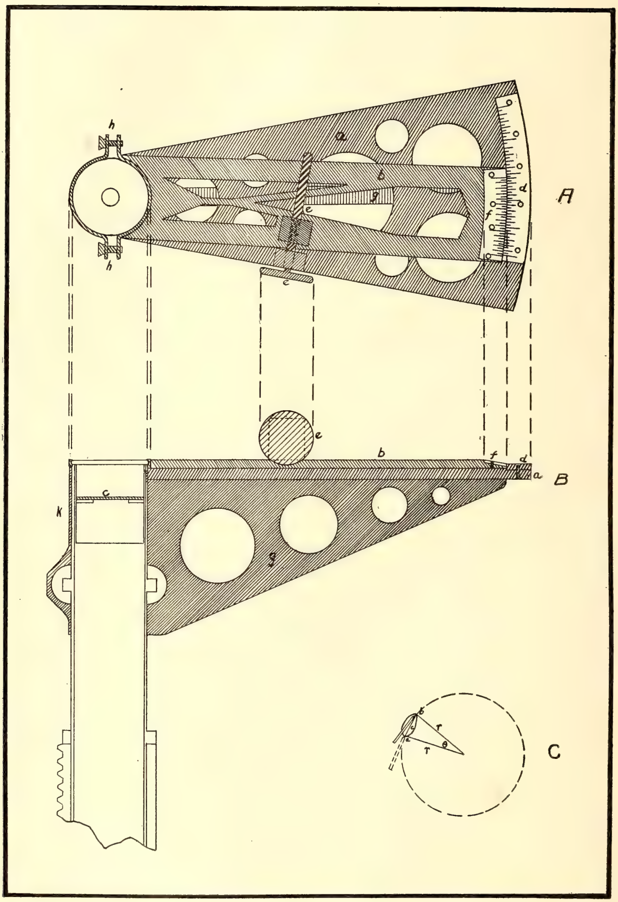
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FIG. 1. The angular micrometer. *A*, a top view of the micrometer in place on the microscope. *B*, a side view. *C*, construction for the calculation of the chord, *c*, formed by the pointer-hair moving from position *a* to position *b*.

The vernier arm is clamped to the microscope and remains fixed during the progress of any measurements. The protractor-arm, *b*, is clamped to the ocular so that by turning the screw, *e*, attached by swivel joints to *a* and *b*, the system, vernier-ocular, is rotated about the common center. In making a measurement the tip of the line in the ocular is brought against the edge of the image formed by the object to be measured, as in figure 2, *c*. The protractor is then read with the aid of the vernier to minutes of arc. The ocular and protractor are then rotated about their common axis until the tip of the line in the ocular appears at the outer edge of the image. The position of the protractor is again read and the difference between the two readings is the θ of the equation given above.

The other quantity in the equation which must be known in order to determine x is the radius, r. Since the line in the ocular is fixed in its position relative to the axis of the microscope, one determination of r is all that need be made for each system of lenses. To determine this radius a suitable scale is placed on the stage of the microscope and θ is determined for some convenient interval of the scale, as described above. The value of the interval in units of length is substituted for x in the equation, the corresponding value of θ, as read from the protractor, is introduced, and r is determined algebraically. This value of r may then be used, with the same system of lenses, for future measurements.

The micrometer is constructed of an alloy of aluminium which is very rigid, is light enough to avoid tipping the microscope tube, and whose coefficient of thermal expansion is very low. This alloy, known as a "White Metal" mixture, is composed of 92 percent aluminium and 8 percent copper. The graduated members, vernier and protractor, are of coin silver and must of course be as accurately graduated as possible with very fine lines. The screw with its milled head is fixed to a swivel block attached to the vernier-arm and works in a threaded bearing attached to the protractor-arm by another swivel joint. This screw may be dispensed with, although it adds to the ease with which delicate adjustments may be made and reduces the pressure on the vernier-arm during the time of making these adjustments. Pressures of the hands in moving the vernier tip the microscope tube, and this changes the path of light through the lenses, the focus, and the apparent position of the object to be measured. This adjusting screw should be affixed as close as possible to the microscope-tube in order to maintain the center of gravity of the instrument near the axis of the tube, and, to increase the ease of manipulation, the milled head of the screw should be large.

The glass slip, *c*, may be either cemented to the annular disc in the ocular and remain there permanently, or it may be cemented to a short tube closely fitting the ocular but removable. The first method is preferable, since it maintains *r* constant for a given objective. This arrangement does not interfere with the ordinary use of the ocular because the line is very fine, very short, and in no way confuses the vision; almost the entire field remains unobstructed and a slight rotation of the ocular changes the position of the line. In default of a photographed line, one may be scratched on a thin, circular cover-slip with a fine cambric needle; such a line will serve equally well, since only its tip is used and the straightness and slope of the line are of no importance. The tip should be sharp and distinct because the exactness with which it may be placed against the object-image controls the accuracy of all measurements. The first micrometer that has been constructed on this plan was built at a cost of $23.00. This instrument however did not possess the adjusting screw, *e*, which would of course add to the cost. On the other hand, because various alterations in the design of the instrument were found necessary during the progress of the work the figure given should include a large part of the cost of the adjusting screw.

The errors to which the angular micrometer is subject by reason of its construction may next be considered.

I. FAILURE TO CENTER PROTRACTOR AND VERNIER

Case 1: Centers of protractor and vernier on a line at right angles to the zero radius of the vernier. (See construction in figure 2.) In the figure the circle *A–A′* is that of the fixed scale upon which the angles are read. *B–B′* is the circle traced by the protractor as the pointer in the ocular moves across the image of the object to be measured. The angles β_1, β_2, β_3 are the angles as read, being those through which the pointer in the ocular apparently moves. The angles γ_1, γ_2, and γ_3 are the true angles corresponding to the first mentioned, and are those through which the pointer in the ocular actually does move. The center of the vernier, *a*, and the center of the protractor, *b*, lie on a line perpendicular to the zero radius of the vernier *a–d*.

In any triangle formed by the intersections on the vernier of the radii of the protractor and the vernier, as *a–c–b*: $180° - (180° - \beta) - \gamma = \alpha$; hence $\beta - \gamma = \alpha$ and

$$\frac{\sin \alpha}{\sin \gamma} = \frac{n}{R}, \quad \text{or} \quad \sin \alpha = \frac{n}{R} \sin \gamma.$$

We may assume that $n = 0.1$ mm. represents the extreme case, for it is hardly conceivable that an error as great as this could be made by any instrument maker likely to be intrusted with the construction of the micrometer. R will be assumed to be 17.5 cm., which is the radius of the instrument now in use. If the degrees are read clockwise from the line passing through *a* and *b* the following relations will be true:

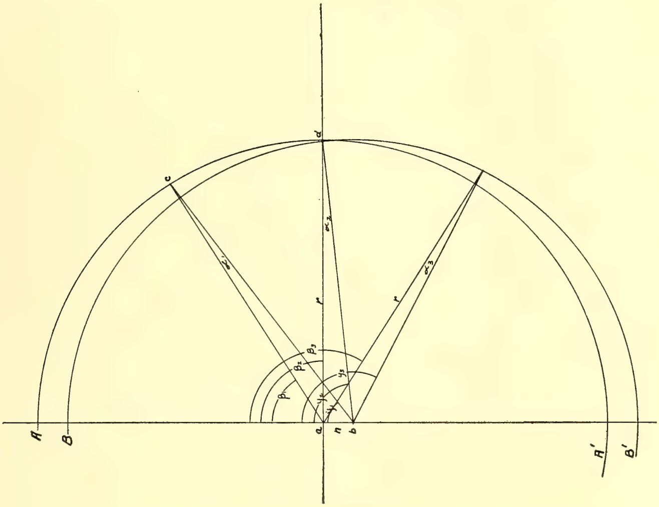

FIG. 2. Construction for errors caused by non-centering of the protractor and vernier.

When $\gamma = 0$, $\sin \gamma = 0$, $\sin \alpha = 0$, $\alpha = 0$ and there is no error.

When $\gamma = 90°$, $\sin \gamma = 1$ and $\sin \alpha = n/R$.

When $\gamma = 180°$, $\sin \gamma = 0$, $\sin \alpha = 0$, $\alpha = 0$ and there is no error. It is apparent that the error is a maximum when $\gamma = 90°$, and in that case $\sin \alpha = n/R$, and since $n = 0.1$ mm. and $R = 17.5$ cm., $\alpha_2 =$ a little less than $2'$. That is, the difference between the observed angle and the true angle will be a little less than $2'$ of arc.

Since a $15°$ protractor has been found to have sufficient range for all ordinary purposes, the illustration may be made more applicable to actual conditions if an angle of ten degrees is considered. Suppose this angle to be measured between $80°$ and $90°$ and thus at the point of maximum error. For both $80°$ and $90°$ $\beta > \gamma$, and hence $(\beta_2 - \beta_1) - (\gamma_2 - \gamma_1) =$ the angular error. $\beta_2 = \gamma_2 + \alpha_2 = 90°\ 2'$; $\alpha_1 = (n/R) \cdot \sin 80° = 1°\ 57'$; $\beta_1 = \gamma_1 + \alpha_1 = 80°\ 1'\ 57''$; $\gamma_2 - \gamma_1 = 10°$; $\beta_2 - \beta_1 = 10°\ 3''$. The angular error is, then, $3''$.

The linear error, y, is found from the equation

$$2r \cdot \sin \frac{\beta_2 - \beta_1}{2} - 2r \cdot \sin \frac{\gamma_2 - \gamma_1}{2} = y.$$

With the combination of lenses (No. 2 ocular and 1/12 oil-immersion objective) with which the micrometer has been chiefly used, $r = 0.030885$ mm. When this value is substituted in the equation y is found to be 0.000001 mm., a negligible quantity. Furthermore, y will decrease with increasing magnification because r, which decreases with increasing magnification, enters the equation as a factor.

Case 2: Line joining the two centers on the zero radius of the vernier. In principle this is case 1. An angle of $10°$ measured between $0°$ and $10°$ would be the same as an angle of $10°$, between $80°$ and $90°$, of case 1. The same conclusions hold for both.

II Failure to Have the Radii of the Protractor and Vernier the Same

Only the case in which the vernier radius is longer than that of the protractor need be considered. The reverse would cause the protractor either to bind in turning or to overlap the vernier, and either difficulty would at once be apparent. If the end lines of graduation of vernier and protractor lie on the same straight line and both members are accurately graduated, the angle read will be the true angle regardless of the length of the protractor radius. If they do not lie on the same straight line the error may be detected by inspection.

III. FAILURE TO GRADUATE ACCURATELY THE VERNIER AND PROTRACTOR

Case 1: Spacing on either scale unequal. If the protractor and vernier are used at different points on their scales and the ratio between the length of the spaces on the two scales is not constant, the error is readily detected by the failure of the end lines of the vernier to cover invariably the same distance on the protractor. Suppose the vernier to be so graduated that thirty half-degree spaces on the protractor equal twenty-nine spaces on the vernier. A slight displacement of the twenty-ninth line of the vernier from the prolongation of the thirtieth on the protractor would be evident and hence a variation of even one minute of arc would be immediately perceived.

Case 2: Spaces on the protractor not accurately half-degrees. This error is impossible of easy detection, but it in no way alters the accuracy of the instrument for it is necessary only that each interval be equal to every other interval; their absolute magnitude is a matter of complete indifference. This is true because all the constants used in the calculations depend upon a known magnitude, which is used for the calibration of the instrument.

IV. FAILURE TO FIX THE PLANE OF THE VERNIER PERPENDICULAR TO THE AXIS OF THE MICROSCOPE

(It is only necessary to consider the vernier, since its position determines that of the protractor.)

Case 1: The angle between any radius drawn on the surface of the vernier-carrier and the axis of the microscope is the same for all radii. This is equivalent to shortening the radius of the protractor, because the common center of both circles is still on the axis of the microscope although in a lower plane. The results of this error are the same as for source II.

Case 2: The angle referred to under case 1 is not the same: the plane of the vernier is tilted from the horizontal. If the tilting is great enough to bring about even 0.5 mm. difference in level between the highest and the lowest portions of the vernier, it may be readily detected by laying a straight edge upon the vernier-arm, when in position flush with the open end of the microscope tube. If the straight edge is then moved over the surface of the vernier-carrier a very slight space between the two will be plainly evident. Suppose, however, that the difference in level is 0.1 cm. The construction for this source of error is shown in figure 3, in which c is assumed lower than b by a distance of 0.1 cm. and b lies in the plane a–b–d which is perpendicular to the axis of the microscope. Since this is the plane in which the protractor should move, the angle a–d–b is the true angle which should be used in all calculations. The angular error is obviously the difference between angle a–d–b and angle a–d–c.

Let angle a–d–b = 15°, the maximum angle which may be read from the protractor of the micrometer now in use. Let b–c be drawn perpendicular to the plane a–b–d and be equal to 0.1 cm. Let $R = 17.5$ cm. Then $ab = 2R \cdot \sin 7^\circ\ 30' = 4.568$ cm. In the triangle b–a–c the angle a–b–c = 90°. Hence, $bc/ab = \tan b\text{–}a\text{–}c = 0.1/4.568$ cm., and $bac = 1^\circ\ 15'\ 14''$. Since $bc/\sin bac = ac$ and $\sin (adc/2) = ac/2R$, then $adc/2 = 7^\circ\ 30'\ 9''$. It must be concluded that the error is negligible for even this extreme case as the instrument is sensitive only to minutes.

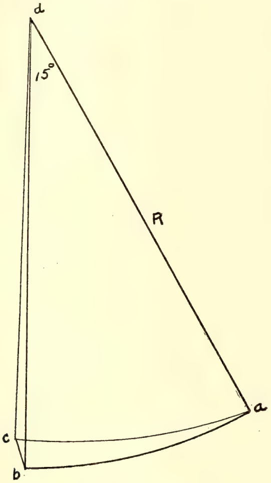

FIG. 3. Construction for the error caused by tilting the vernier.

V. ERRORS CAUSED BY TILTING THE OCULAR IN ITS SLEEVE

Since the protractor-carrier rides upon the vernier-arm and since each has a broad surface of contact with the other, there would be no tilting of the ocular even if it fitted rather loosely in its sleeve.

VI. ERRORS CAUSED BY THE LATERAL PLAY OF THE OCULAR IN ITS SLEEVE

This may best be detected by setting up the instrument with the tip of the line in the ocular at the edge of some object in the field, and then attempting to move the ocular back and forth in its sleeve by pressure upon the vernier-arm. The microscope should be securely clamped during the process as it is important that the axis of the microscope should not be tilted at the same time. With the ordinary close fit of the oculars of standard makes there is no alteration of the apparent position of the pointer hair.

VII. Errors Caused by Tilting the Microscope Axis

To detect this error, remove the micrometer and close the substage iris-diaphragm until it forms a pin-point aperture and substitute for the ocular the pin-point cap, provided with the microscope. If the eye is now applied to the opening in the cap a beam of light will be perceived if the condenser and the lenses are in alignment as they should be for any detailed microscopic work. If the condenser is not centered, it should be adjusted until a beam of light reaches the eye through the pin-hole aperture. The micrometer should now be attached and the process repeated. If the micrometer tilts the tube the beam of light will no longer be perceived and the micrometer should be counterpoised. This is usually not necessary if the rack-and-pinion coarse adjustment fits as tightly as it should to prevent the tube from gradually sinking during the progress of the measurements and producing focal errors.

For obvious reasons the sources of possible inherent error have been considered somewhat at length, and although each instrument must be examined for error, it must be apparent that, assuming only reasonable skill in instrument construction, the number of actual errors in any instrument is small and that, moreover, they are all easily detected. Since the accuracy of any instrument of precision depends not only upon the theory of its construction and the care and skill with which this theory is realized in the instrument itself, but also upon the care and skill with which it is used, methods for avoiding those errors of practice which are generally known to be the most important may be briefly stated in conclusion.

The calibration of the angular micrometer, as of all ocular micrometers, requires the knowledge of the absolute distance between two fixed points on a suitable slide. The commercial stage micrometers have been found to be almost useless under high magnification, not only because of their variation in spacing but also because of the width and irregularity of their lines. The following method was accordingly devised and has been found to be very satisfactory. These scale errors are significant only at high magnifications, and r may be determined with a good photographed scale on a stage micrometer under low magnification with great accuracy. Having determined r for this magnification, another object should now be substituted for the ruled scale. This object should possess two points that are well defined under both this and a higher magnification. When the distance between these points is determined r may be found for a higher power with which the original ruled scale would be useless. It is thus possible to proceed from lower to higher powers, substituting new objects when necessary until the desired magnification is reached. Although the method is a little tedious, it is no more so than the routine of ordinary accurate mensuration and is much more satisfactory than the use of photographed scales with the higher powers.

The presence of the micrometer of course alters the tube-length and it is universally known that this must be maintained approximately that for which the lenses were ground. Ashe[3] has devised a very easy and accurate method for determining the tube length experimentally. The correct tube length D may be found in millimeters from the equation $(B \cdot C)/(A - B) = D$, in which A represents the number of spaces of a stage micrometer visible in any certain interval of an ocular micrometer when the tube length is D. B represents the number of such spaces visible in the same interval of the ocular micrometer when the tube length is drawn out beyond D by a distance C, expressed in millimeters.

Inaccurate focusing may be the cause of large errors, and it has been found that the weight of the microscope and the micrometer may in the course of half an hour or less, depending upon the frictional resistance of the coarse adjustment, cause such errors in the focus that large variations in measurements result. Because distinctness of image is not a reliable criterion for judging the focus,[4] I have modified a method devised by Cornu and Benoît[5] in such a way that it is applicable for use with microscope slides and is useful for very slight errors of focus. Figure 4 illustrates this method. If a beam of light from a radiant point *O* on a plane mirror is directed through the narrowed orifice of an iris diaphragm and strikes the condenser at one side of the center, the light will pass through the microscope along the path *O–A–F*. If directed through the opposite side of the condenser, by moving the diaphragm, the beam will traverse the path *O′–B–F′*. It is evident that if an object is placed on the stage in such a position that the pointer-hair in the ocular apparently touches its edge, a movement of the diaphragm will be followed by a movement of the image past the pointer-tip if the object is out

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[3] Ashe, A., Note on the determination of "optical tube length." Journ. Quekett Micros. Club, Series 2. **5**: 152. 1892.

[4] See: Apathy, Stefan von, Microtechnik. 2te Abt. Leipzig, 1901. 300 pp., and Stephenson, J. W., Observations on Prof. Abbe's Experiments illustrating his theory of microscopic vision. Month. Microsp. Journ. **17**: 82–88. 1877.

[5] Cornu, A., and J. R. Benoît, Rapport sur la détermination de l'Étalon provisoire International. Bureau International des Poids et Mesures. Trav. et Mém. **10**: 12. 1894.

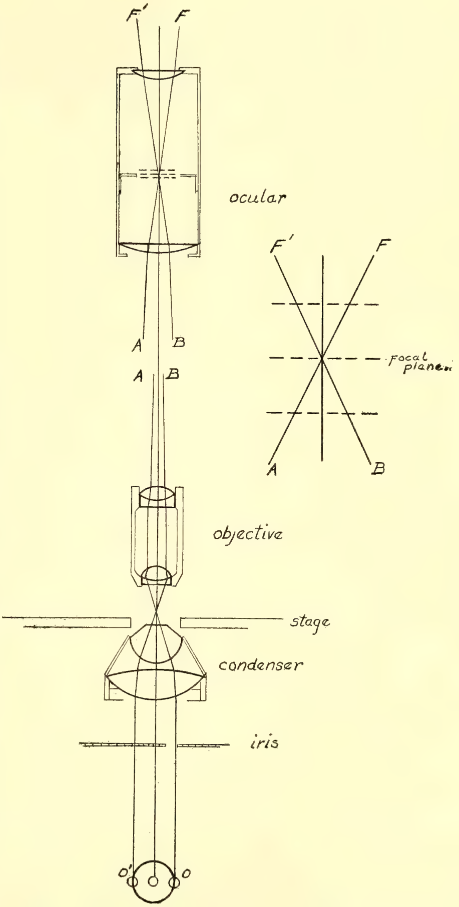

FIG. 4. Method for detecting focal errors. By means of the iris-diaphragm a beam of light is made to pass over either path *O–A–F* or *O′–B–F′*. If the image in the microscope remains stationary the object is in focus.

of focus. An object will, accordingly, be in focus only when there is no movement of the image as the diaphragm is moved back and forth. It is unnecessary, except for completeness, to direct attention to the importance of maintaining a "critical illumination."[6]

With a Leitz No. 2 ocular and a 1/12 oil-immersion objective, the angular micrometer as constructed has a sensitiveness of 0.000009 mm., *i. e.*, a movement of the protractoı through 1′ of arc would move the pointer-tip over the image of an arc whose chord is 0.000009 mm. The use of higher powers increases the sensitiveness as does also placing the pointer-hair near the periphery of the field. Both are obviously unnecessary so far as sensitiveness is concerned.

Farmer and Digby[7] have observed that it was impossible for them to measure chromosomes with an accuracy greater than 0.0001 mm., and this number may be taken as the limit of accuracy of former methods of measurement. Measurements were made with an angular micrometer upon an individual somatic chromosome of Chinese Lily, during anaphase, with about half the magnification of the English authors, and consequent loss of accuracy. They were made at different times on different days, so that it is believed they offer a fair test of the efficiency of the instrument. In nineteen measurements the maximum deviation from their mean was 0.00003 mm. and the difference between the extremes was 0.00006 mm. The maximum variation is therefore about six-tenths that found by Farmer and Digby, and would of course be still further decreased with increased magnification since this would increase the accuracy with which pointings might be made.

It is a pleasure to acknowledge my indebtedness to Dr. C. E. Allen for his help and interest.

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[6] See the discussion in some good work on the microscope such as Spitta, E. J., Microscopy. London, 1907. 468 pp.

[7] Farmer, J. B., and L. Digby, On dimensions of chromosomes considered in relation to phylogeny. Phil. Trans. Roy. Soc. London, *B*, **205**: 1–25. 1914.

INFLUENCE OF THE MEDIUM UPON THE ORIENTATION OF SECONDARY TERRESTRIAL ROOTS

RICHARD M. HOLMAN

In a recent paper[1] I called attention to the inadequacy of the explanations put forward by earlier investigators for the striking difference in the behavior of primary roots which have been diverted from their normal position while growing in air or water, on the one hand, and earth, sand or other non-fluid medium, on the other hand. My experiments led to the conclusion that, after the primary root in air or water has flattened the primary geotropic curvature, a considerable resistance on the part of the medium to the advance of the root tip is a necessary condition for a subsequent complete curvature of the root. After the flattening of the primary curvature, the root in air grows straight ahead in an oblique position. Although the increasing length and weight of the root may result in its reaching the perpendicular through bending under its own weight, active reaction to the stimulus of gravity is generally restricted, after the completion of the autotropic flattening, to the extreme tip. This tip curvature, although varying in intensity, is maintained as long as the root is well supplied with water, is actively growing and has not attained an approximately perpendicular position. In such media as earth, sand, sawdust, and sphagnum, which offer more or less resistance to the root's advance, a root with such a curvature of the tip curves downward into the normal position in a curve whose radius is smaller the greater the resistance offered to the advance of the root. This downward curvature is apparently due to a passive depression of the root resulting from the non-symmetrical application (relative to the axis of the root) of the force opposing the advance of the root tip. These conclusions, arrived at as the result of a study of the behavior of the primary roots of *Vicia faba*, *Lupinus albus*, and *Pisum sativum*, naturally suggested the question, whether or not the secondary roots of these species showed any relation between geotropic behavior and medium such as exists in the case of primary roots.

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[1] The orientation of primary terrestrial roots with special reference to the medium in which they are grown. Amer. Journ. Bot. 3: 274–318. 1916.

In the course of the experiments upon the curvature of primary roots in different media, I had noted that the secondary roots arising from the upper side of a primary root, which had been planted horizontally in loose moist sawdust or just below the surface of very loose soil, showed little tendency to bend downward. They frequently bent so slowly that they emerged from the medium and grew obliquely upward into the moist air above the cultures for several centimeters. In such cases they never re-entered the medium from which they

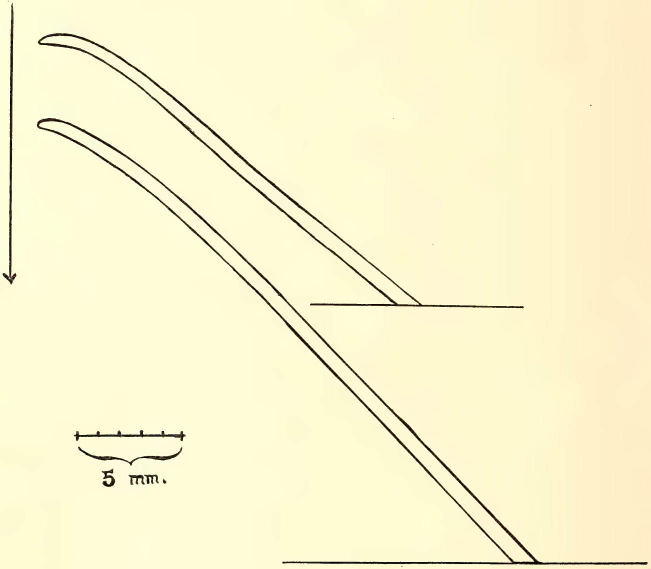

Fig. 1. Secondary roots of *Vicia faba*, which arose from a primary root planted horizontally near the surface of fine moist soil. After emerging from the soil the roots grew upward into the air as shown in the figure.

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[2] Compare Sachs, Ueber das Wachsthum der Haupt- und Nebenwurzeln. Arb. Bot. Inst. Würzburg. 1: 633. 1874. Here Sachs reports the emergence of secondary roots from the soil when the mother root was growing perpendicularly. The secondary roots arose from the hypocotyl but those of which I speak had their origin 1 to 4 cm. below the root base.

had emerged.[2] Figure 1 shows camera drawings of two such roots. Of these roots, the upper one had grown 2.2 cm. after leaving the soil (the surface of which is represented by the horizontal lines) and the lower one 3.2 cm.

Sachs[3] is the first author who reported any comparative observations of the behavior relative to gravity of secondary roots in different media. His principal observations were, 1st, that the limiting angle (Grenzwinkel) of secondary roots growing in air was greater than that of roots growing in water and greater in roots growing in the latter medium than in the case of secondary roots growing in earth, and, 2d, that, in air, the relatively acute curvatures resulting from displacement of the roots upward from the limiting angle or from subjecting them to a stimulus greater than gravity, by means of the centrifuge, were later flattened. Sachs employed principally seedlings of *Vicia faba*. Relative to the first of Sachs' observations, stated above, Czapek[4] reported that wetting of relatively dry sawdust cultures resulted in an increase, rather than a decrease, in the limiting angle of secondary roots growing in the sawdust. This result was just the opposite of what Sachs had reported when dry soil cultures were abundantly wetted. Both Czapek and Sachs stated however that the behavior of roots under the conditions mentioned was very inconstant. Sachs's own statement[5] that secondary roots in air which are not frequently wetted lose their ability to bend downward suggests that the roots which showed a smaller limiting angle in air than in water may have behaved thus on account of too low humidity of the air surrounding them.

My own experiments with *Vicia faba* var. *equina* and var. *major* and with *Lupinus albus* and *Pisum sativum* disclosed a striking parallel in the behavior of primary roots and secondary roots of the first order. *Vicia faba* was used for most of the experiments because of the large number of secondary roots which it produces and the great vigor of growth which they display. (There are usually five rows of secondary roots in the case of *Vicia faba*, two rows in the case of *Lupinus albus* and three in the case of *Pisum sativum*.) The media used were air, maintained as nearly as possible at the point of water saturation, uniformly moist sawdust, and fine-sieved garden earth. In some cases the primary roots were decapitated before the appearance of the secondary roots (15 centimeter roots were cut to 11 or 12 centimeters), in other cases the main roots were not decapitated. In any single experiment, however, all the seedlings were treated alike. Compact sawdust was not employed as in the case of experiments with primary roots because the secondary roots always followed a very sinuous course in that medium as did also the slender primary roots of *Vicia sativa* L., *Ervum lens* and other species. This was no doubt due to the mechanical weakness of the roots and the resistance of the particles of the sawdust (which were considerably larger than those of the soil used) to the advance of the root tip. In loose sawdust and in soil, neither the very irregular curvatures which took place in compact sawdust nor the slight "wellenförmig" curvatures which Sachs[6] observed in

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[3] L. c., 609 ff.

[4] Czapek, Ueber die Richtungsursachen der Seitenwurzeln und einiger anderer plagiotroper Pflanzentheile. Sitzungsb. Akad. Wiss. Math. Naturw. (Wien) **104:** 1 Abt: 1253. 1895.

[5] Sachs, l. c., p. 609.

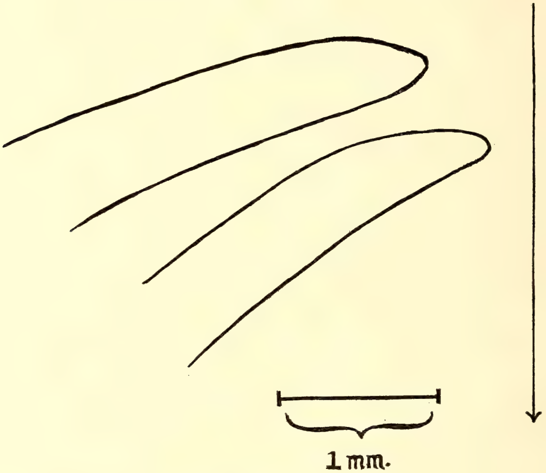

FIG. 2. Two secondary roots of *Vicia faba* var. *equina* which arose from a primary root planted horizontally in loose moist sawdust and grew obliquely upward through that medium, maintaining a curvature of the extreme tip.

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[6] Sachs, l. c. p. 611.

soil cultures made their appearance. In view of the change of geotonus of secondary roots which Sachs,[7] Stahl[8] and Czapek[9] have reported as accompanying temperature changes, all the cultures in a single experiment were kept at the same temperature. During the course of all the experiments the temperature varied from 15° to 19° C. The cultures were kept in darkness except for short periods during which observations, drawings or photographs were being made, because, as Czapek[10] has shown, the geotonus of the secondary root changes when the root is illuminated. All cultures employed in a given experiment were exposed to light for the same length of time when the behavior of the roots was recorded.

When secondary roots growing in air were so displaced that they formed a greater angle with the normal direction of the main root than their limiting angle, a curvature followed which, as Sachs has stated, involved the whole growing region. I also found, in accordance with Sachs's observations, that this curvature was subsequently considerably flattened and thereafter further permanent curvature was very slight or entirely lacking unless the root was still far removed from its limiting angle. The roots, however, maintained after the flattening of the first curvature a curvature or strong asymmetry of the terminal 1 to 1½ mm. The retention of this curvature or asymmetry of the tip was clearly dependent upon the maintenance of the turgor of the cells of the tip, for it could be caused to disappear and reappear repeatedly within a few minutes by alternately exposing the root to relatively dry air and spraying it with water. This curvature of the tip which is maintained by the root, although there may be scarcely any or indeed no permanent curvature, is shown in figure 2. It is similar to the curvature of the tip of the primary root which Němec[11] first reported. As in the case of the primary root, this curvature of the extreme tip of the secondary root does not pass over as a permanent curvature to the region behind the tip, but is continually being flattened and at the same time is being renewed by the cells of the growing point and the root cap.

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[7] Sachs, l. c. p. 624.

[8] Stahl, Einfluss des Lichtes auf den Geotropismus einiger Pflanzenorgane. Bericht. Deutsch. Bot. Ges. **2**: 396. 1880.

[9] Czapek, l. c. p. 1251.

[10] Czapek, l. c. p. 1250.

[11] Němec, Ueber die Wahrnehmung des Schwerkraftreizes bei den Pflanzen. Jahrb. Wiss. Bot. **36**: 93ff. 1901.

In water the behavior of the secondary root was, in my experiments, very much the same as in moist air, although growth persisted much longer than in air and as a result the roots soon became so long that they bent downward under their own weight. There was only slight active and permanent curvature of the roots in water except when they formed a very large angle with the normal position of rest.

In media offering resistance to the advance of the root, the behavior of the secondary root was directly parallel to that of the primary root. Roots which had grown for a time in the limiting angle in loose moist sawdust curved very gradually toward the normal position when displaced upward from the original position, whereas roots growing in earth and similarly treated curved promptly and acutely into a position approximately the same as that from which they were displaced. This difference in behavior is represented in figure 3, which was traced

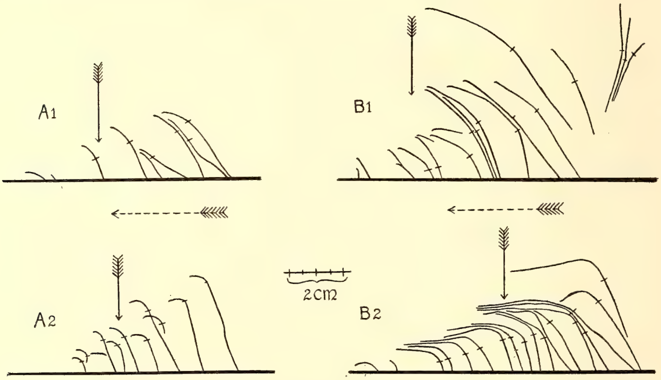

FIG. 3. Curvatures in loose moist sawdust and in earth of the secondary roots of *Vicia faba* when displaced 90° from the normal position. *A*1 and *B*1 represent roots growing in loose sawdust; *A*2 and *B*2, roots growing in earth. The arrows with dotted shaft show the direction of gravity relative to the root system at the time the secondary roots were of the length indicated by the cross strokes on the roots. It was at that time the cultures were turned through 90°. The arrows with solid shaft indicate the direction of gravity after the cultures were turned. Roots below the main root are omitted. Roots in *A*1 and *A*2 grew for the same length of time in both positions; also roots in *B*1 and *B*2.

from photographs of roots in earth and loose moist sawdust cultures. Within a few hours after they had been displaced from their limiting angle, the roots in both media showed a very distinct curvature of the tip which was more distinct in the case of roots in loose sawdust than in the case of those in earth. As the roots in the former medium continued to grow, the curvature of the tip was continually flattened behind and was constantly reformed at the very extremity of the root, as in the case of roots in air. The flattening was not, however, complete and there remained a slight permanent curvature. In some cases this permanent curvature was so slight that it was perceptible only after the root had elongated three or four centimeters. The curvature of the tips of roots in soil was almost completely fixed and as the roots elongated an acute permanent curvature resulted which brought the root tip into a position not much (5°–20°) removed from the former limiting angle.[12] Thus a secondary root in earth generally reached within 24 hours at 15° to 16° C. a position which was attained by a root in loose sawdust only after 3 to 5 days. The secondary roots on the lower side of a horizontally placed main root often grew for 2 or 3 days in either of the media without showing any tendency to curve upward. This was due apparently to two factors. The first of these is the relatively slight angle these roots formed with the normal position of the secondary roots. The second is the still unexplained tendency of secondary roots which are displaced downward from their limiting angle to react less promptly and intensely than secondary roots which have been equally diverted above the normal position of rest.[13] The downward directed secondary roots, however, curve more promptly upward when growing in earth than when they are in loose sawdust.

The very close parallel which exists between the behavior of secondary roots under stimulus of gravity in different media and primary roots under the same conditions indicates that the part played by the medium in both cases is the same. After flattening of the primary curvature mechanical resistance to change in the root's form and to the advance of the root is necessary to complete reaction and within certain limits the greater this resistance is the more promptly the reaction is completed. As in the case of the primary roots, it is probably by hindrance of the flattening of the curvature of the root tip and by passive depression of the curved tip as it is pushed forward through the medium that the resistance of the medium reinforces and renders complete the geotropic reaction.

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[12] Sachs (l. c. p. 627) noted the increase of the bounding angle following each curvature.

[13] Czapek, l. c. p. 328 ff.

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THE ANATOMY AND PHYLOGENETIC POSITION OF THE BETULACEAE*

Carl S. Hoar

According to the system of Engler the Betulaceae are placed together with the Fagaceae in the cohort Fagales, which in turn is assigned to a position near the bottom of the Archichlamydeae. At the beginning of the Archichlamydeae is found the cohort Verticillatae, containing but one family, the Casuarinaceae. The Casuarinaceae are represented by a single genus, Casuarina, and the species of the latter occur in the Australasian and East Indian regions.

In this work it is my purpose to treat anatomically and phylogenetically the family Betulaceae. First, however, in order better to understand the situation with regard to this family, it seems best to treat somewhat in detail the family Casuarinaceae which is in close anatomical and phylogenetic relationship with the above.

The position of these lower families, as has been stated, is the one given by Engler and generally followed. However, since the origin of the Angiosperms is still somewhat in doubt, opinions with regard to the true evolutionary position of the various lower members of the Dicotyledons naturally vary. Evidence for one view is favored by one organ while perhaps another may give just as convincing evidence for an opposite conclusion. It is only when everything is taken into consideration that one can arrive at a proper understanding of the true situation.

Among the various opinions there are two in particular which stand out from the others. The first is that of an origin from the Cycadales, while the second is one from the Coniferales through the Gnetales. Both of these views were based until recently almost exclusively upon the reproductive structures. Of late, however, Thompson (1912) has undertaken a study of the Gnetales with the vegetative anatomy as his object and has already published his results as found in the genus Ephedra. His research brings forward evidence for the coniferous origin of the Angiosperms rather than for a derivation from the Cycadales. To quote directly from the author's published account: "The idea of cycadalean and bennettitalean affinity receives little support from the anatomy of Ephedra. On the other hand, there are many points which are opposed to it and in favor of the coniferous relationship; the arrangement of the primary vascular bundles, double leaf-traces, arrangement and structure of the pits on the tracheides, bars of Sanio, tertiary spirals, trabeculae and resin plates, primitive uniseriate lignified rays, wood parenchyma, and endarch vascular bundles of the leaf. The Gnetales do not appear to have arisen from any modern group of Conifers, but rather from or close to the base of the coniferous line."

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* Contribution from the Laboratories of Plant Morphology of Harvard University.

"An angiospermous affinity is indicated by the possession of true vessels, broad rays, and separation of the leaf-traces upon the stem." Figure 1 shows a tangential view of the broad (aggregate) ray in *Ephedra californica.*

Among others who have held to the idea of the gymnospermous affinity of the Angiosperms we find Eichler (1878) stating that the Amentiferae are derived directly from certain gymnospermous families. Treub (1891) and others were also of this opinion.

The next question to arise is which of the families of the Angiosperms are lowest and hence most closely allied to the Gymnosperms. Here again a diversity of opinion prevails. Eichler (1890), according to the account of Moss (1912), considered Casuarina as belonging to the amentiferous forms and as not having peculiarities striking enough to warrant a separation from this group. Engler (1897) himself so placed the genus until Treub's work appeared in 1891. Treub claimed that certain reproductive characteristics made it necessary to consider the Casuarinaceae as lower than the Amentiferae proper. Since that time the family has been placed by Engler's system in the Verticillatae at the very bottom of the Dicotyledons. Warming (1912) justifies this position in the following words: "By the transfusion tissue, by the type of the stomata, and especially the ovular peculiarities, the order seems to be related to the Gymnosperms, especially the Gnetales." With regard to the ovular peculiarities, he found that "in the nucellus, which has two integuments, many embryo-sacs are formed, of which only one is fertilized. The egg-apparatus consists, as among the Angiosperms, of an egg-cell and two synergidae; but antipodal cells are not formed. Before fertilization a prothallus of many free nuclei is developed which after fertilization becomes a nutritive tissue. The fertilization is chalazogamic." These remarks according to Moss (1912) are based upon Treub's work. Moss, however, goes on to say that Frye (1903) has shown Treub to be wrong in regard to the anomalous embryo-sac, large number of nuclei, etc. He remarks that all of these characters suggested by Treub have been found in *Carpinus* and many of them in *Corylus*, *Betula* and *Alnus*. These are such as chalazogamy (fertilization of the egg-nucleus through the chalaza rather than direct as in the case of the majority of the Angiosperms), the occurrence of more than one embryo-sac, the formation of a coecum from the originally isodiametric embryo-sac, the occurrence of tracheides among the embryo-sacs, the entrance of the pollen tube at the base of the coecum in Corylus and Carpinus, the closure of the micropyle, and finally the fusion of the ovule with the wall of the ovary. Frye's results are corroborated by the work of Benson (1894), who showed that chalazogamy not only prevails among the Casuarinaceae but also among the Betulae and Coryleae, and that Castanea as well as Casuarina possesses tracheides around the base of the embryo-sac. Again, Benson (1906), in collaboration with Sanday and Berridge in 1906, found still further evidence that Casuarina differs in no essential from others of the Amentiferae. All genera, as she remarks, contain arborescent, wind-pollinated species. All are monoecious with flowers closely aggregated in unisexual catkins. The female flower in all is dimerous, bearing free stigmas, and the ovary of all genera is inserted in a radial plane. Casuarina differs from Carpinus exactly as does Quercus from Fagus, and the absence of a perianth in the female flower of Casuarina is a character common to Betula and Alnus. She states that the most important features of difference are its switch-like character and its phyllotaxy. These combined with some minor differences in floral structure and in embryology may suffice as grounds for forming a distinct group within the Betulaceae equivalent to the Coryleae. Thus he would dispense with the term Verticillatae and leave out such a cohort in the classification of the Dicotyledons.

From the above brief statement the reader may observe that the actual phylogenetic position of the Casuarinaceae remains at present somewhat in doubt. Although, as stated above, it is now placed in a cohort of its own at the very base of the Dicotyledons, yet there are recent data to show that it is really in no important respect distinct from the Betulaceae and other amentiferous plants. As will be noted, the work in this case, as well as in the case of the Gnetales, has been for the most part confined to the reproductive structures with no special reference to the vegetative bodies. Let us now turn to a somewhat brief survey of the latter structures in Casuarina. As work is now being carried on in this laboratory with regard to the above, it is not necessary to enter into a detailed discussion, but several points may be emphasized.

In studying the anatomy of a group of plants in order to determine their evolutionary relationships it is especially desirable to know and to be able to interpret their wood structure. Recent work on the Gymnosperms has proved of great assistance and one is now able to classify them more nearly in a true phylogenetic order. Little work has as yet been done upon the Angiosperms; but if the principles used in the case of the Gymnosperms are valid, they should hold equally in the case of the Angiosperms.

With this in mind we may turn to the anatomy of the Verticillatae. The wood, here, contains vessels which are small in diameter. These have, even when in contact with the wood parenchyma, bordered pits. Their end walls may often have simple, elliptical perforations such as are characteristic of the more highly developed Angiosperms, but these, in every case examined, are accompanied by the scalariform type characteristic of the lower forms. In some species the vessels also have spiral tertiary thickenings. Wood parenchyma is quite abundant and is always found scattered throughout the entire annual ring in the same position (diffuse) as it occurs in the higher Gymnosperms. With regard to the wood prosenchyma the walls are usually thick and possess the bordered pits characteristic of Gymnosperms.

It is in connection with the rays that the most interesting condition is found. Among the Coniferales the rays are uniseriate except in the case of those which contain horizontal resin canals. This condition is not prevalent among the Dicotyledons, or even among the Gnetales, although some uniseriate rays are present. Later it is my purpose to trace the evolutionary development of the higher types of rays but it will now be advantageous simply to state the situation as it exists in the Casuarinaceae. Certain species, such as *Casuarina torulosa* (figure 2), show around the leaf-trace a congestion and grouping of the rays into a large, "false ray,"—the aggregate type. This character persists throughout the entire growth of the plant. In other forms a different situation occurs. *Casuarina Fraseri*, for instance, shows in the primitive regions a ray quite like the type found throughout the wood of *Casuarina torulosa*. In maturity, the woody elements have been entirely lost and the small rays have become completely fused into an exceedingly large one. Such a ray is called compound. There is still another type, that found in *Casuarina equisetifolia* and *Casuarina stricta*. Here the rays in the first annual rings are in the aggregate stage. When the tree is old enough, a different condition from that found in *Casuarina Fraseri* occurs. The rays do not fuse together to form one large ray. Instead, as the aggregate type passes into the outer rings of growth, there is a tendency to separate, or, in other words, to become diffused into small rays two or three cells in width. If a large section is examined, this diffusion will be seen to take place in a perfectly diagrammatic manner. Thus Casuarina is a generalized type, as appears when one looks at the matter from the standpoint of the ray formation.

Turning from the Casuarinaceae let us consider the amentiferous forms in order to obtain some idea of the position which they occupy in the evolutionary scale. At present the Betulaceae and Fagaceae are placed in one cohort called the Fagales. This cohort is allocated by Engler eleventh from the base of the Dicotyledons. Since there is much uncertainty as to the exact relationship of these lower cohorts, the placing of the Fagales in the eleventh position simply means that the group belongs near the base of the Dicotyledons. Until recently the study of these forms, as in the case of the Casuarinaceae, has been confined largely to the reproductive organs. From such studies great diversity of opinion has arisen and no definite conclusions have been reached. In order that the reader may obtain some idea of the apparent confusion which has resulted, it seems desirable to give a brief account of the situation.

The peculiar floral apparatus, the ament, has caused much uncertainty upon the part of observers. A large group hold that the floral structure is not a primitive but a reduced form. Van Tieghem (1868), as far back as 1868, after making a study of Juglans (belonging to the cohort Juglandales) and of the Coryleae, claimed that their floral anatomy was similar to that of the higher Angiosperms. Prantl (1887), in his study of the Cupuliferae, looked upon the Fagaceae and the Betulaceae as derived by reduction from plants with bisexual flowers, possessing a perianth, multilocular ovary, and suspended ovules. He considered that the two families have been developed independently, the Fagaceae being the more primitive of the two (a view not held by all). Hallier (1901 and 1908) attempted to demonstrate the affinity of the Cupuliferae and the higher Angiosperms. In doing so he considered the Cupuliferae as derived from the Hamamelidaceae and through these from the Laurineae. In this way he would connect them with the Rosalean forms allied to the Combretaceae. It is noteworthy in this connection that in 1908 he discarded the view held in his earlier paper and considered the above as derived from the Anacardiaceae and Burseraceae chiefly because of the strong anatomical likeness of Juliana to those orders upon the one hand and to Juglans upon the other. Clearly the observer had very small proof upon which to base his belief and hence, perhaps, his reason for changing his opinion. Goebel (1905), through his work, came to the conclusion that the Fagales and Juglandales are reduced forms. The superior gamophyllous perianth, syncarpous ovary, and complicated inflorescence, he thought not characteristic of a primitive family. Arber and Parkin (1907) also took issue with those who consider the same cohorts as not being reduced forms. Recently Berridge (1914) observed that the Amentiferae are not an isolated group but have an obvious relationship with the higher Angiosperms. In her article, the inflorescence, flowers and cupule, of *Castanopsis chrysophylla* are described and the anatomical structure of the flower is fully worked out. This species is compared to *Castanea vulgaris*, *Fagus sylvatica*, *Quercus Robur*, and *Juglans regia*. She found the flower differing in no essential from the epigynous types of the angiospermous flowers and drew a comparison between the Rosaceae and the Cupuliferae, since it seemed probable to her that the epigynous Rosaceae, or their near descendants, are the forms with closest affinity to the ancestors of the Fagaceae.

Among those who hold a different view upon the subject we find such men as Treub and Engler. Since those who believe in the reduction theory have worked freely with the Juglandales, it is well to note the work of other investigators upon the same group. Nawaschin and Finn (1913) worked with *Juglans regia* L. and *Juglans nigra* L. and came to the following conclusions: Among seed plants there is the tendency to reduce the male gametophytes from sperms to naked nuclei. Together with this reduction occurs the evolution of the pollen tube. The species of Juglans studied have binucleate generative cells which reach the embryo-sac without disorganization and which nearly correspond to the binucleate generative cells of certain Gymnosperms. In this feature, therefore, they conclude that these species occupy an intermediate position between the Gymnosperms, in which the male cytoplasm reaches the egg-cell, and the higher Angiosperms in which the male cytoplasm disorganizes in the pollen-tube and even in the pollen-grain. Hence they look upon the persistence of the male cytoplasm in Juglans as a primitive character retained from their gymnospermous ancestors; and, moreover, consider that the appearance of this character in chalazogams is significant and is a further proof of the great age of these plants. The tendency in seed plants to reduce the male gametes goes hand in hand with the evolution of the pollen-tube and seems correlated with its appearance. Such an opinion is the opposite of the one held by Van Tieghem (1868) in his work upon the floral anatomy of Juglans and the Coryleae. Nicoloff also (1904) came to an opposite conclusion from that of Van Tieghem and supported the views of Nawaschin. He considered the Betulaceae, as did Nawaschin, to be derived from the Coniferales while he would derive the Casuarinaceae from the Gnetales. Before discussing the above views let us note the opinion given by Coulter and Chamberlain (1903). Their position is one which does not bind them to either side. They state that none of the writers who regard the Amentiferae as derived from the Angiosperms with bisexual flowers suggest an affinity with any group of the Archichlamydeae but seem rather to incline to the author's opinion; namely, that whether they represent a single genetic stock or several, they appear to be isolated from the higher alliances.

By means of the short account just given in regard to the evolutionary position of the Fagaceae and the Betulaceae, one may readily understand that from the study of the reproductive structures alone the situation has not been well cleared up. Perhaps the position taken by Coulter and Chamberlain is the one which it is safest to assume when the above field alone is taken into consideration. However, some interesting facts may be pointed out from what has already been said concerning Casuarina. If, as appears to be the opinion of some, the Casuarinaceae are so closely allied to the amentiferous type of plants as to be almost, if not quite, worthy of being placed with them instead of in a group by themselves, then how can the latter group be considered as one which has undergone any great reduction?

To be sure there may be indications of reduction in some instances with regard to the reproductive structures, but on the other hand, such investigators as Nawaschin have found good grounds for supposing that there are very important primitive features in connection with the formation of the male cell which lead to a very different conclusion. That Juglans should show a condition part way between the Gymnosperms and the higher Angiosperms would appear to constitute a strong point against the contention that any great reduction has taken place. Moreover, the vegetative anatomy of the Casuarinaceae is most certainly primitive. Nearly every organ in this family shows primitive features although they are sometimes accompanied by characters common to the higher forms. Granting the close relationship to the Casuarinaceae it does not seem possible to regard these amentiferous forms as belonging anywhere but low in the evolutionary scale of the Dicotyledons. There are, also, other points which are, perhaps, best mentioned here. The species of all these families are characterized by having the egg fertilized from the lower side through the chalaza. Such a type of fertilization, as has been stated, is commonly known as chalazogamy in contrast to the type found throughout all the Monocotyledons and higher Dicotyledons. This latter type is called porogamy. In the latter, also, the pollen-tube grows down the style of the pistil and instead of following the wall of the ovary, as in the case of the chalazogamous forms, grows directly through the intervening space and into the egg-sac by way of the micropyle. The chalazogamous type is confined, among the Dicotyledons, to the amentiferous forms in general and is plainly not of high evolutionary character. We find an instance of a similar condition among the Gymnosperms in the Araucarineae. Here, in the case of the genus Araucaria, the pollen falls upon the ligule. It does not grow directly down to the micropyle but follows the tissues of the ligule and scale until it attains a position immediately below the ovule. Upon arriving at this point, it grows straight upward through the lower side of the megasporangium and reaches the egg from the bottom. In the chalazogams the pollen falls upon the stigma and the pollen tube grows down through the solid tissue into the egg. Apparently the situation in those members of the Dicotyledons characterized by chalazogamy is intermediate between the case in the Gymnosperms and that in the higher Angiosperms. The grains of pollen no longer fall upon the ovule but the condition has not yet been reached where they grow through space and thus fertilize the egg by way of the micropyle.

Another feature worthy of mention is the way in which these plants are pollinated. Among the Dicotyledons there are two chief agencies for carrying this out. All of the higher members of the group are pollinated by insects. Such plants usually possess well developed floral envelopes. On the other hand the Betulaceae, Fagaceae, and Casuarinaceae together with other families of the lower Dicotyledons and all of the Gymnosperms are not pollinated in this way. Pollination in these takes place through the agency of the wind. This has long been recognized as a characteristic common to the lower families of the Angiosperms. If these forms are reduced to any great extent, they would be expected to show some trace of insect pollination. An instance may be mentioned of a family which is even now placed very low because of its apparently simple floral structure but which is pollinated by insects. This family is that of the Salicaceae belonging to the cohort Salicales. Miss Holden (1912) has worked with these and, since the principles which she has used are the same as those which are being used here, it will not be amiss to give in some detail a summary of her results. Through recent work it has been shown that the presence of wood parenchyma and its distribution are very definite things and hence of great value in determining the evolutionary relationships of a plant. In the lowest Gymnosperms no wood parenchyma is present. When it first occurs, it appears only at the end of the annual ring (terminal) and is evidently developed in correlation to the alternation between summer and winter in connection with storage of food material. The next step, and one which is common to the higher Gymnosperms and the lower Angiosperms, is the diffusion of the wood parenchyma throughout the entire annual ring. Among the higher Angiosperms the wood parenchyma is more or less localized around the vessels. In Salix the parenchyma is terminal. This condition by itself is deceptive, since not even the higher Gymnosperms are so characterized. Again the rays throughout our common species of Salicaceae tend to be uniseriate like those of the Conifers. Because of this structure and by reason of the apparently simple flower this family has been placed at or near the bottom of the Dicotyledons.

Miss Holden in making her study used exactly the same principles which have led to such excellent results among the Gymnosperms.

These may be catalogued under three heads—recapitulation, retention, and reversion. The principle of recapitulation is of much importance in the case of plants. Very often the seedling gives evidence of an ancestral condition which no longer prevails in the mature form. The principle of retention, similar to that of recapitulation, is particularly characteristic of plants. Scott first brought this to light in connection with the peduncle of the Cycads. More recently this principle has been greatly extended and it is now known that certain organs and regions of the plant often retain ancestral conditions lost in the more advanced parts. Thus the root throughout all plants is characterized by centripetally developed primary wood which now does not appear in the stem of any forms above the zoidogamous Gymnosperms. Other parts which often manifest retention are the reproductive axis, the first annual ring of the vegetative or reproductive stem, the leaves, etc. The last principle, that of reversion, has been shown of late to be likewise of great importance. In this way conditions which are no longer present in our living forms may be recalled as a consequence of injury. In the consideration of the Betulaceae the validity of the general principles cited above will be assumed.

Of the two families classified by Engler in the cohort Fagales, the Fagaceae are generally thought to be the higher. This family consists of several well known genera, among which may be mentioned Quercus, Fagus, Castanea, and Castanopsis; and some of the anatomical peculiarities of these are here described. The genus Fagus is usually divided into two sections, namely, Eufagus and Nothofagus. The species of Eufagus (northern beeches) possess broad rays, while those of Nothofagus (antarctic beeches) have rays of only one to two cells in width. In addition to the distinctions of ray structure the wood fibers of Eufagus have bordered pits in contrast to those of Nothofagus in which the pits are simple. In the latter also the fibers are sometimes septate. Castanea and Castanopsis show close agreement with regard to the wood. Narrow medullary rays occur extending outward from the angular pith. A feature of difference from the two genera last named is the presence in Quercus of *broad* medullary rays.

The second family of the cohort Fagales and the one dealt with especially in this work is the family Betulaceae. It includes the following genera: Alnus, Corylus, Carpinus, Ostrya and Betula. In the anatomy of their woods certain general features occur. Narrow medullary rays are present which are but one or two, rarely four, cells in width, the cells being mostly elongated in the radial direction and containing clustered crystals. The vessels have small lumina, are arranged in radial rows, bear bordered pits in contact with the ray parenchyma and always have scalariform perforations. Wood parenchyma is present and is not usually arranged in the plates so characteristic of Quercus. The wood prosenchyma or tracheids have wide lumina and bordered pits, which are not numerous and whose border is distinctly smaller than the pit itself. The above statements apply to the members of the Betulae (Alnus and Betula). Among the Coryleae (Carpinus, Ostrya and Corylus) there are many features in agreement with the Betulae. The medullary rays are small, usually being from one to two, or sometimes three, cells in width. The wood prosenchyma is characterized by wide lumina and bordered pits; and the vessels are always arranged radially with small lumina. However, where two vessels join each other, the walls bear densely packed and rather large bordered pits, the margin often assuming a hexagonal contour. Again, where the vessels come in contact with the ray parenchyma, their pits are almost entirely simple. There are spiral thickenings in some members. In all investigated species of Carpinus and Ostrya the perforations of the vessels are, for the most part, simple and elliptical, and the scalariform type is confined largely to the primary wood. Corylus, however, does have exclusively the scalariform type. The Coryleae in general have more wood parenchyma developed than do the Betulae and it is often found in bands as is characteristic of Quercus.

The preceding paragraph will afford an idea of the main features of the Betulaceae from the standpoint of wood structure. It is chiefly from the evolutionary aspect that they will be treated in this work. From this point of view, as in the case of the oaks, the rays show very interesting and important conditions.

Alnus, a genus ranging in stature from the arboreal to the fruticose, is generally distributed throughout the north temperate zone, and is abundant in eastern North America and in eastern Asia. Its rays vary much in size and degree of development according to the species. Bailey (1912) has already shown this in some detail. So far as his work goes, it has been possible to verify his results. Certain forms, as *Alnus acuminata*, show practically no tendency towards aggregation of the ray. Instead, the rays, even in the mature stem, are usually uniseriate. This is not a feature characteristic of the whole group; but is rather the exception. Take, for example, our common form, *Alnus incana* L. Here the entirely uniseriate rays contrast with apparent aggregations of rays. In the latter the slender component rays are separated from one another by fibers only; the vessels characteristic of the remaining wood structure being conspicuous by their absence. *Alnus rugosa* Du Roi shows a condition very similar to that of *Alnus incana* and therefore needs no special description.

In the case of *Alnus japonica* a somewhat different situation is present. In figure 3 is shown part of the woody cylinder of a three-year-old branch of this species. Vertically in the center is seen the aggregation of rays corresponding to a leaf-trace, terminated below by the trace itself and subtending the leaf-gap. In the aggregation of rays, vessels are clearly absent. On either side of this central congery of rays lies the wood which has the ordinary structure except where smaller aggregate rays are present. The latter are the dwindling remnants of foliar aggregations belonging to nodes higher or lower in the stem.

Figure 4 is a tangential view under about the same magnification, taken from the stem of the same species. In the center near the top may be seen the round cluster of cells which makes up the leaf-trace and which pass out horizontally to the axis of the tree. Extending for a short distance above this and for a much greater distance below, a noticeably larger clustering of elements may be observed, differing from the surrounding tissue by the absence of vessels. Careful examination shows it to consist of groups of rays (one to three cells in diameter) and separated by tracheids only. In other words, it is an aggregate ray formed about the leaf-trace for the more abundant storage of food.

Various other alders show this tendency to aggregate still further carried out. In *Alnus rubra* the condition is quite like that of *Alnus japonica* with the rays gathered together and becoming more or less multiseriate. Still further compounding appears in *Alnus maritima* Marsh. It may be inferred from the above description that the alders illustrate as regards ray organization a relatively low condition.

Interesting situations are presented by *Alnus mollis* Fernald, a species of northern range commonly found throughout eastern Canada and the Eastern States, and only reaching southward along certain streams which have brought the seeds down in their current. In these southern limits of distribution, the species is much smaller in size than when found in its natural habitat. Figure 5 shows a transverse section of the center of a twig in the region of the node. In the first annual ring a leaf-trace appears on the right with which is associated a vestigial aggregate ray.

This species affords an excellent example of retention and of recapitulation. The large rays, which have disappeared in the ordinary wood, appear again in the first annual ring at the node and around the leaf-traces. Figure 6, a transverse section of the wood of an old stem, shows that even in maturity it does not manifest aggregate rays. Wounding, as illustrated in figure 7, brings about a different situation. In the center several clustered rays occur, though in the regions lateral to these the ray structures are all uniseriate. In figure 8, a transverse section of a mature root, several aggregate rays appear in the outer rings and two such rays run completely through the field.

In the study of this species several salient facts may be noted. In the first place, in the normal stem the region of the internode does not normally show aggregate rays. When it is cut in the region of the node, aggregate rays appear to some extent within the first annual ring and around the leaf-traces. An examination of the seedling root shows its rays to be all uniseriate. Yet, around the root-trace and somewhat after wounding, the aggregate ray reappears, and in the old root it is a permanent feature.

Apparently *Alnus mollis* is a peculiar type, which, after starting to form aggregate rays, has reverted to the uniseriate condition. It is a well known fact that plants in a state of reduction often have ancestral structures in the mature parts. Thus, although the root of the seedling exhibits no large rays, since they have reverted to the uniseriate condition, in the root of the mature plant the aggregate ray reappears. The stem manifests a similar situation. The peduncle of the ovuliferous ament often presents aggregate rays in considerable numbers. The same condition is illustrated by the axes which support the peduncles, and, in fact, not infrequently in the early annual rings of any branches of vigorous development.

Figure 9 is an illustration of the first few annual rings of a twig of *Alnus Yasha*, a Japanese species. Here again, although the mature stem shows no large rays, yet, as the section indicates, in the first annual ring and around the leaf-traces aggregate rays make their appearance. Obviously the species has suffered degeneracy in a degree somewhat similar to that of *Alnus mollis*.

The genus Corylus is characterized by having aggregate rays throughout the entire stem. Figure 10 is a section of the root of *Corylus americana* Walt. cut through the region of the root-trace. It will be noticed that around the trace the rays are aggregated. Figure 11 is that of a longitudinal section of *Corylus rostrata* Ait. taken just below the root-trace. Only ray cells and tracheids with wood parenchyma cells appear extending through the center. It is the aggregate type which is formed about the leaf-trace or the root-trace. The genus Corylus is thus characterized by having the aggregate type of ray well developed in both root and stem.

In the genus Carpinus the situation is comparable to that of Corylus. Figure 12 is that of a transverse section of *Carpinus cordata* cut near the region of the leaf-trace. The rays are here clustered into more or less definite congeries in which the vessels are very scarce. In other words, the wood normally has rays in the aggregate condition. In figure 13, a transverse view of the root of the same species, the rays are in general diffuse but aggregated in relation to the root-trace. Figure 14 is a greater magnification of a portion of figure 13 showing more distinctly the area directly around the root-trace and better illustrating the same characteristics.

In the case of Ostrya the normal stem does not have the rays distinctly aggregated, but instead they are more or less scattered. Nevertheless, as figure 15 indicates, the aggregate condition is somewhat clearly shown in regions near the pith. This is another example of the retention of ancestral characters in the first annual ring. Figure 16, a transverse section of a root of *Ostrya virginiana* Mill., illustrates aggregation (bottom of the figure) and absence of this phenomenon in the rest of the periphery. Figure 17, a higher magnification of the lower part of the preceding photograph, shows the aggregate ray developed in relation to a secondary root. Here may be seen manifested the characteristics of aggregation; namely, the clustering of rays with the exclusion of vessels. The root, most conservative of plant organs, naturally reproduces the primitive condition, aggregation of rays in the vicinity of the appendages. In the stem, contrariwise, we have to do with the most progressive and variable of organs and as a consequence we are not surprised to discover the absence of aggregate rays in its mature structure. Vestiges of these, however, occur, as shown in figure 15, in the earlier annual rings and nearer the pith. In other words, the stem of *Ostrya virginiana* has passed through the aggregate condition and is now characterized by the possession of the diffused type of ray in its mature organization.

In the case of the Betulae there are extremely interesting facts. In *Betula populifolia* Marsh. aggregate rays are characteristically present more or less throughout the whole plant. Because it has the aggregate ray present everywhere, this species must be regarded as one of the most primitive of the birches. On the other hand, *Betula lenta* L., the black or cherry birch, appears to represent a higher degree of development; for in the normal stem aggregation is conspicuously absent. Figure 18 shows a transverse section of *Betula lenta* cut through the root near the root-trace. The two aggregate rays may be plainly seen clustered about the region of the outgoing traces. Again it will be noted that the root illustrates the retention of the ancestral characters of the plant. The European white birch, *Betula alba* L., and its variety the canoe birch, *Betula alba* var. *papyrifera* Marsh., likewise supply interesting data. Figure 19 is that of a transverse section of the stem of *Betula alba* cut from a small twig at the very top of the tree. In the center of the field a very noticeable and large aggregate ray is visible. Such rays are characteristic of the vigorous upper catkin-bearing branches of this species. Ancestral structures are well known to occur in reproductive regions; and the situation presented by the species under consideration affords a further illustration of this important general principle.

Figure 20 shows a transverse section of a seedling twig of *Betula alba*, var. *papyrifera.* It has been wounded at about the third annual ring and again near the end of the fifth year. As a result of the wounds the structure has reverted and has formed many large aggregate rays. This is another instance of the traumatic recall of ancestral types, a condition very common throughout the Conifers. Figure 21 shows a small portion of figure 20 enlarged. It is clear that the majority of the rays are from two to three cells in width, *i. e.*, of the diffused type. Upon either side, however, is a large aggregate ray which has come in subsequent to the wounds shown in figure 20.

Figure 22 is that of a young twig of the same species cut in the vicinity of the leaf-trace. In the normal stem cut from the internode no large rays appeared. Nevertheless, around the lateral leaf-traces a tendency to aggregate occurs in the first annual ring and somewhat in the second, but beyond these only the diffused type is present. Figure 23 is a transverse section of a twig cut from the extreme top of an old and vigorous tree and illustrating a condition similar to that shown in figure 19. Aggregate rays are not noticeable in the mature vegetative stem of this species except in relation to the leaf-trace and in the first annual ring. They are conspicuously present for a number of years in the seedling stem of *Betula alba* (*sensu strictiore*), but are not found even in the first annual ring of the seedling of *B. alba*, var. *papyrifera*. The *wounded* seedling of the variety does, however, show aggregate rays strongly developed as a result of injury. The vigorous catkin-bearing branches of this species likewise often manifest the aggregate ray clearly present, and figure 24 illustrates the interesting conditions which are found in the root. On the upper side of the figure appears a denser region of the wood increasing in breadth from the primary wood outwards. This is an aggregation of rays related to the trace of an outgoing secondary root.

Conclusions

On the whole the genus Alnus most clearly illustrates the conditions which are apparently primitive for the Betulaceae. In *Alnus japonica*, for example, we find congeries of somewhat enlarged rays related to the appendages (roots, leaves and lateral branches). These clusters of rays present in several features a contrast to the general ray conditions in the wood. First of all, the members of such ray groups are individually lengthened or broadened or both in contrast to the uniseriate organization of the rays of the mass of the wood. Secondly, they show more or less pronounced tendency to fuse with one another. Thirdly, the vessels which characterize the longitudinal structure of the normal wood are conspicuous by their absence in the grouping of rays under discussion. This condition of organization is well illustrated by *Alnus japonica* as figured in plate I. In other species of *Alnus* the tendency to grouping or aggregation of rays with the concomitant peculiarities described above becomes obsolescent, being retained only in regions of greater conservatism and susceptible to recall as a result of injury. *Alnus mollis*, *Alnus rugosa*, *Alnus incana*, *Alnus maritima*, etc., etc., show more or less marked degeneracy in these particulars.

In the genus Corylus the condition is simple. As figures 10 and 11 indicate, there is a distinct tendency towards aggregation.

In the genus Carpinus we apparently have a very interesting situation. Here in most species the wood structure is characterized by rays not uniseriate as in Alnus, but two or three cells wide and separated from one another by the usual longitudinal elements, namely, vessels, fibers and wood parenchyma cells. In contrast to this organization of the wood which may be justly considered to be normal, we find clustered rays not opposed as in *Alnus* to those of the general wood structure but distinct because of their clustered condition as well as by reason of the absence among them of the longitudinal elements belonging to the category of vessels. It has been made clear at the beginning of this article in connection with the genus Casuarina that the type of dicotyledonous wood characterized by the uniform distribution of rays not uniseriate but usually of mediocre breadth (two to many cells) is the result of the diffusion of rays originally grouped, throughout the woody cylinder. The special interest of Carpinus is that it presents this condition and that of aggregation from which it has been derived, side by side. In *Carpinus cordata* aggregate rays are found only in the earlier annual rings, disappearing in the adult. On the other hand, in *Carpinus caroliniana* and *Carpinus betulus* the aggregate and diffuse ray conditions persist together in the mature structure.

The wood organization of Ostrya is very similar to that of *Carpinus cordata.* In the mature wood of the stem grouped rays have entirely disappeared, but vestiges of them may be discerned in the earlier annual rings adjacent to the pith. The aggregate rays also persist in this genus in the root.

The genus Betula is of peculiar interest on account of the large number of species and the considerable variety of wood structure which they present. In the common gray birch, *Betula populifolia*, of the Eastern States, we find diffuse rays and aggregations of rays close together. In *Betula alba* the congeries or aggregations of rays are found in the first annual ring of the vegetative stem, during a number of years in the seedling stem, and even more strikingly in vigorous reproductive branches. Aggregations of rays are likewise a feature of normal structure in the root and are related to the secondary roots. In *Betula papyrifera* (*Betula alba*, var. *papyrifera*) the occurrence of aggregations of rays is much more restricted. They are absent in the seedling, but are normally found in the root. They may be recalled in the seedling stem though not in the adult as a consequence of injury. In *Betula lenta* and *Betula lutea* the condition of aggregation is clearly present only in the root. It is thus apparent from this summary that in the genus Betula the phenomenon of aggregation of rays is a primitive condition persisting with the diffuse type in *Betula populifolia*, but in other species confined more or less definitely to primitive organs and regions or recalled only as a result of injury.

In a recent article an attempt has been made to discredit the aggregate ray as the precursor of the conditions of ray structure obtaining in the mass of arboreal Dicotyledons (Bailey and Sinnott, 1914). In the present connection it can only be emphasized that the conclusions which are logically drawn from the facts must be in accord with the general principles established as a result of the comparison of fossil (Mesozoic and Paleozoic) Gymnosperms with those still living. It is certain from these studies that ancestral conditions persist strongly in reproductive structures and in roots, and that they may be present in the earlier rings of growth of the vegetative stem even when absent in the mature wood. Further, they often reappear in response to injury or abnormal situations. Applying these principles to the Betulaceae from Alnus to Betula, beyond reasonable question the phenomenon of aggregation of rays is primitive for the family. The genus Alnus illustrates this type in a primitive condition. In the higher genera, Carpinus, Ostrya and Betula, it has given place to the diffuse state resulting from the lateral spreading of the original congeries or aggregations of rays; but in these genera the ancestral condition often persists in conservative organs and regions or may return as a consequence of injury. If the principles here cited are sound and the conclusions drawn are logical, the criticism above referred to appears to have very slight value.

It seems clear that the anatomy of the cohort, of which the Betulaceae form one member, is distinctly that of a low group. The presence of parenchyma distributed throughout the entire annual ring is a feature not usually found in the higher Dicotyledons. The vessels, also, with their characteristically scalariform perforations are unquestionably of a primitive nature. The fibers are in general fiber-tracheids, and above all, the rays show features of organization which are common to the lower families of Angiosperms and to the highest Gymnosperms. With these facts in view, it is obviously impossible to assign this family to a very high position.

Summary

1. That, although the floral anatomy of the Betulaceae may give indications of a reduction instead of primitiveness, yet there are other facts which must be taken into consideration.

2. That whether the Verticillatae should or should not be separated from the Amentiferae is not a question of great importance from our standpoint.

3. That the structure of the Verticillatae is either entirely primitive or so generalized as to include both primitive and advanced conditions, thus indicating that the cohort belongs close to the base of the dicotyledonous line.

4. That the closeness of anatomical relationship between the Verticillatae and the true Amentiferae gives evidence as to the primitiveness of the latter.

5. That the Betulaceae in general show clear evidence of a primitive aggregation of specialized rays in relation to the appendages, and that these rays become subsequently diffused throughout the structure of the wood.

6. That the alders in general present the aggregate condition either normally developed or in a state of reduction.

7. That in the higher members of the Betulaceae (Carpinus, Ostrya and Betula) the aggregate condition clearly lies in the evolutionary background, persisting only in conservative organs and regions or recalled by injuries.

8. That the general internal anatomy of the Betulaceae and especially the ray structures supply no proof for and much against their being placed anywhere but near the base of the Dicotyledons.

9. That, finally, unless some new and more conclusive facts to the contrary are brought to light, the Betulaceae must be ranked in a low phylogenetic position.

In conclusion, the writer wishes to express his thanks to the authorities of the Arnold Arboretum for the privilege of collecting material with which to carry on this work; and, also, to those who have in any way aided. This work has been done in the Phanerogamic Laboratories of Harvard University under the direction of Prof. E. C. Jeffrey.

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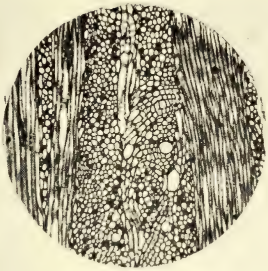
EXPLANATION OF PLATES XVI–XIX

FIG. 1. *Ephedra californica.* Tangential section of wood showing aggregate ray. × 30.



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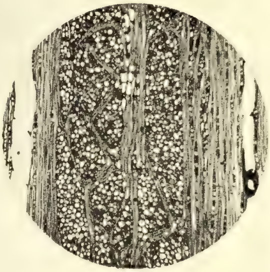

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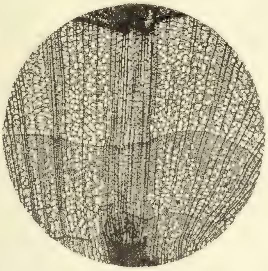

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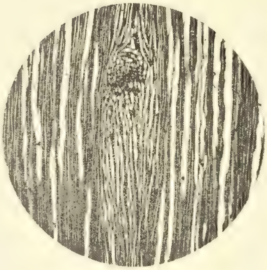

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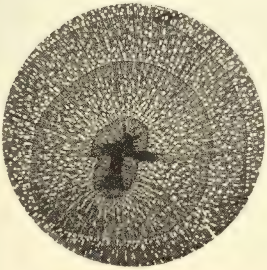

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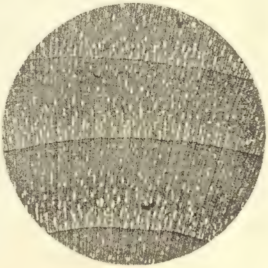

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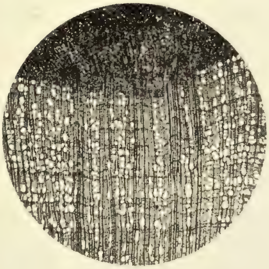
HOAR: ANATOMY OF BETULACEAE.



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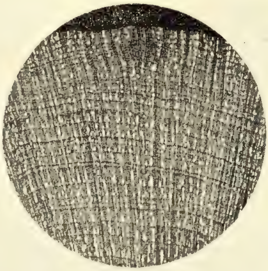

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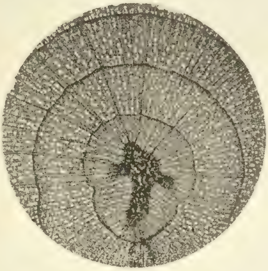

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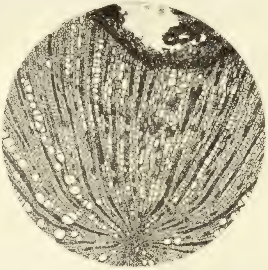

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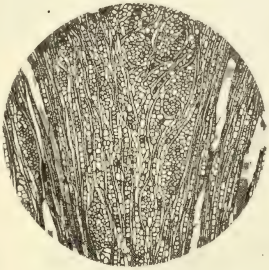

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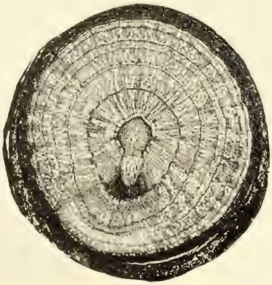

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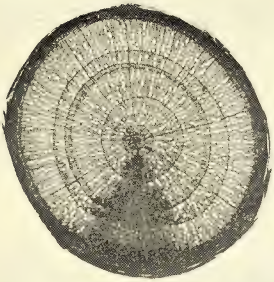
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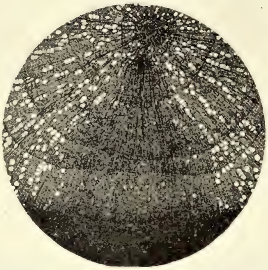
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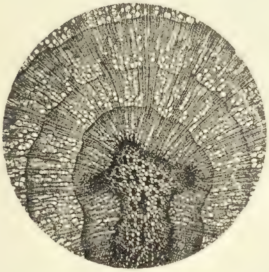
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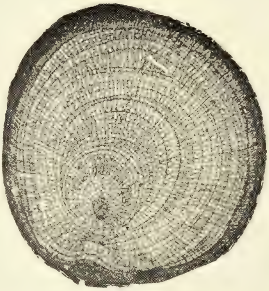
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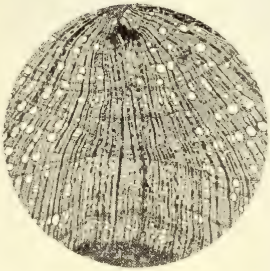
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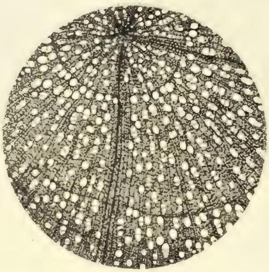
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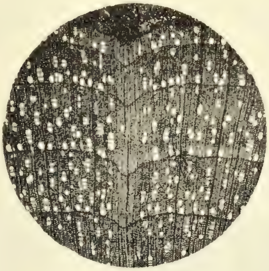

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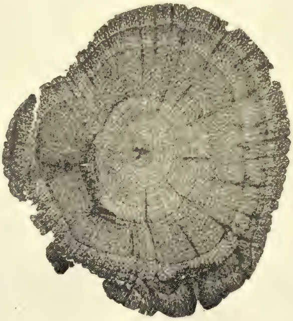

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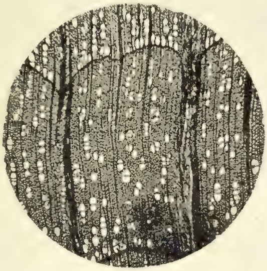

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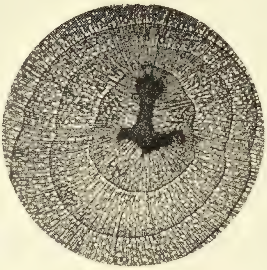

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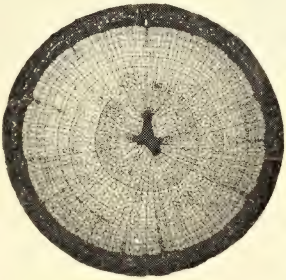

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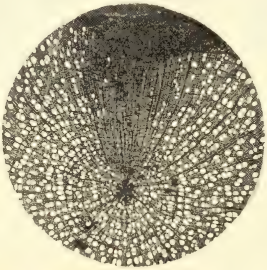

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HOAR: ANATOMY OF BETULACEAE.

FIG. 2. *Casuarina torulosa.* Tangential section of wood showing aggregate ray. × 30.

FIG. 3. *Alnus japonica.* Transverse section of aggregate ray enlarged in vicinity of leaf-trace. × 20.

FIG. 4. *Alnus japonica.* Tangential section through aggregate ray and leaf-trace. × 25.

FIG. 5. *Alnus mollis.* Transverse section of young twig through region of node showing aggregate rays, the largest of which is related to a leaf-trace. × 10.

FIG. 6. *Alnus mollis.* Transverse section of wood of stem showing only uniseriate rays. × 20.

FIG. 7. *Alnus mollis.* Transverse section of wounded root showing aggregations of rays. × 20.

FIG. 8. *Alnus mollis.* Transverse section of part of an old root showing normal aggregate rays. × 15.

FIG. 9. *Alnus Yasha.* Transverse section through the first annual ring of a twig showing aggregations of rays. × 15.

FIG. 10. *Corylus americana.* Transverse section in the region of a root-trace showing aggregation of rays in relation to the lateral root. × 25.

FIG. 11. *Corylus rostrata.* Tangential section cut in the vicinity of the root-trace showing aggregation of rays. × 25.

FIG. 12. *Carpinus cordata.* Transverse section of twig showing aggregations of rays. × 10.

FIG. 13. *Carpinus cordata.* Transverse section of root showing an aggregate ray related to a secondary root. × 15.

FIG. 14. *Carpinus cordata.* Transverse section of the same aggregate ray as in Fig. 13 enlarged. × 30.

FIG. 15. *Ostrya virginiana.* Transverse section through the early annual rings showing a persistent tendency to aggregate. × 20.

FIG. 16. *Ostrya virginiana.* Transverse section of root showing aggregation of rays about the trace of lateral root. (Upper side). × 15.

FIG. 17. *Ostrya virginiana.* Transverse section of aggregate ray around trace of lateral root, much enlarged. × 30.

FIG. 18. *Betula lenta.* Transverse section of root showing aggregation of rays in relation to lateral roots. × 30.

FIG. 19. *Betula alba.* Transverse section of wood of a twig near the top of the tree showing aggregation of rays. × 20.

FIG. 20. *Betula alba* var. *papyrifera.* Transverse section of axis of wounded seedling showing traumatic aggregate rays. × 15.

FIG. 21. *Betula alba*, var. *papyrifera.* Part of Fig. 20, more highly magnified. × 30.

FIG. 22. *Betula alba*, var. *papyrifera.* Transverse section of twig in region of node showing vestigial aggregations related to leaf-traces. × 10.

FIG. 23. *Betula alba*, var. *papyrifera.* Transverse section through reproductive branch showing persistence of aggregate rays. × 10.

FIG. 24. *Betula alba*, var. *papyrifera.* Transverse section of root showing aggregate ray related to lateral root. × 15.

THE TOXICITY OF BOG WATER

George B. Rigg

The following is a brief statement of data obtained by the writer in experiments on waters from sphagnum bogs of the Puget Sound region and Alaska. The flora of these bogs has been previously described (6, 7). In most cases the water was collected by cutting a shallow cavity in the sphagnum and dipping up the water which accumulated within a few minutes. In a few cases during the dry season, water could not be obtained in this way. It was then obtained by squeezing it from handfuls of sphagnum obtained a few inches beneath the surface of the bog. Details of the experiments here reported as well as an account of other work on this water now in progress will be reported later.

1. When they were filtered through filter paper, then saturated with NaCl, $MgSO_4$, Na_2HPO_4, or $(NH_4)_2SO_4$ and allowed to stand over night the samples tested have all shown a precipitate.

2. When this precipitate was filtered off and the filtrate dialyzed in a dialyzing tube in running water until it no longer showed a precipitate with $BaCl_2$, this filtrate did not prove toxic to root hairs on Tradescantia cuttings placed in it, while controls in bog water allowed only a very poor development of root hairs on cuttings of this species.

3. When 500 cc. of filtered bog water was distilled until the residue was only 80 cc., the distillate was colorless and was not toxic to root hairs on Tradescantia cuttings while the residue was much darker in color than bog water and almost entirely inhibited the formation of root hairs on these cuttings.

4. When saturated with $(NH_4)_2SO_4$ and allowed to stand over night the above distillate gave no precipitate while the residue gave a much heavier precipitate than untreated bog water did.

5. All samples of bog water tested were acid to litmus and to phenolphthalein.

6. The acidity of the residue mentioned in 3 was greater than that of the untreated bog water while the acidity of the distillate

was less than that of the untreated bog water. The acidity was determined by neutralization with $N/20$ NaOH, using phenolphthalein as an indicator.

7. The specific gravity of a number of samples of bog water has been tested by weighing them in a specific gravity bottle and has been found to be 1.000.

It has been frequently shown that bog water is injurious at least to certain plants (1, 2, 5, 6, 9). Analyses of Ohio bog water (3) and of water from a lake adjoining the bog indicate that the bog water differs from the lake water in a higher content of organic NH_3 and in greater loss on ignition. It has been shown (8) that the surface tension of bog water is not low enough to account for its toxicity. The fact that the osmotic pressure of bog water is very low (1, 4, 8, 10) suggests that the material in solution in it is probably in a colloidal state. The data here given seem to confirm this view and to warrant the suggestion that this colloidal matter is a large factor in the toxicity of the water.

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ON THE OSMOTIC PRESSURE OF THE TISSUE FLUIDS OF JAMAICAN LORANTHACEAE PARASITIC ON VARIOUS HOSTS[1]

J. Arthur Harris and John V. Lawrence

I. Introductory Remarks

This paper, which is one of a series on various physiological, ecological, phytogeographic, and evolutionary problems involving a knowledge of the physico-chemical properties of vegetable saps, has for its object the presentation of a series of observational data on one phase of the water-relations of tropical Loranthaceae—the osmotic pressure of their tissue fluids in comparison with that of their hosts.

Since our method of attack upon the physiology of the Loranthaceae is so far as we are aware quite novel, it has seemed most expedient to focus attention sharply and practically exclusively upon the actual results of our studies, reserving a detailed discussion of the literature until other work now planned and under way has been completed.

Whatever the answer to the mooted question of the nature of the solutes obtained by the parasite from its host—whether solely mineral or both mineral and plastic—the nature and the magnitude of the forces by which the solution containing these substances is drawn from the tissues of the host is a subject of fundamental importance.

Furthermore, few botanists would, we believe, be inclined to question the validity of the proposition that among these forces one of the fundamental variables is the osmotic pressure of the fluids contained in the tissues of the two organisms.[2]

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[1] Results of investigations carried on at Cinchona, by courtesy of the British Association for the Advancement of Science and the Jamaican Local Government, under the joint auspices of the Department of Botanical Research and the Department of Experimental Evolution of the Carnegie Institution of Washington, and with the collaboration of the New York Botanical Garden.

[2] As far as we are aware the only botanists who have actually published this view are MacDougal and Cannon (1910) and MacDougal (1911*a*, 1911*b*), who considered a higher osmotic pressure in the sap of the engrafted organism to be one of the essentials of experimentally induced parasitism.

We have therefore determined by means of the cryoscopic method the osmotic pressure of the tissue fluids of host and parasite.

II. Materials and Methods

In the late winter and early spring months of 1915, we had the opportunity of spending some weeks in a study of the concentration of the saps of the plants of the Coastal Deserts and of the Montane Rain Forest of the Blue Mountains of Jamaica, splendidly described and illustrated by Shreve (1914). In the latter habitat Loranthaceae are abundant. York (1913) has given a detailed account of the morphology of two of the seven species which we have studied.

The parasites, structurally and physiologically considered, fall into two groups, those with and those without leaves.

The leaves of Phthirusa and Phorodendron may be looked upon as comparable with those of the host. Such can not legitimately be assumed of the green stems of the three (physiologically) leafless Dendrophthora species. In all the determinations based on these species, we had some difficulty in deciding what parts of the tissue of the parasite to include. It would obviously be quite illegitimate to compare the most tender recent growth of the parasite with the matured leaves of the host. It would also be quite wrong to draw the comparison between juices pressed from the oldest stems of the parasite with the leaves of the host tree. Possibly the best method would have been to scrape the green tissue from the outside of the parasite stems, but this would have entailed a very great deal of time and would have exposed the constants to obvious criticisms. Furthermore this method while applicable to *Dendrophthora gracilis* and *D. opuntioides* could not well be used for *D. cupressoides*. We decided, therefore, to include the whole of the obviously mature axis tissue, omitting only the very young growth, when such was present, and the older heavier stems which while once physiologically leaf homologs could not possibly be still so considered.

The technique used was very simple. Samples of the tissue of the parasite and host were collected in test tubes of about 100 cc. capacity and taken to the laboratory for freezing by immersion for several hours in an ice and salt mixture. The sap was then extracted by pressure in a small heavily tinned press bowl with a powerful hand screw. After filtering, the freezing point lowering of the sap was determined by the use of a thermometer graduated in hundreths of degrees with divisions sufficiently large to allow of reading to approximately thousandths of degrees. Ether or carbon bisulphide vaporized by a dried air current in a Dewar bulb was used in determining the freezing point of the sap.

In many instances a cloudiness or flocculent precipitate similar to that described by Gorke (1906) was observed when the sap approached the freezing point or passed it in undercooling. We had no facilities for any investigation of these substances but believe their presence does not greatly, if at all, influence our results.

The results are recorded in degrees depression, Δ, corrected for undercooling, and in atmospheres pressure, P, from a table published elsewhere (Harris and Gortner, 1914).

III. Presentation and Analysis of Data

We were able to make freezing point determinations on seven species representing three genera of Loranthaceae, parasitic upon a considerable number of hosts. Altogether 44 samples of parasitic tissue were used. In 42 cases sap was also extracted from the leaves of the host.

The results, arranged primarily by the species of parasites and secondarily by the magnitude of the freezing point lowering, appear in the following protocol. For convenience of reference our field numbers have been retained. The values of Δ and P found for the parasite are given at the extreme right, opposite the host on which it was taken. Those found for the tissues of the host are placed immediately below the name of the host species. The + and − values given below the constants for the parasite show whether they are larger or smaller than those obtained from the tissue of the host, and the magnitude of the difference.

I. Phoradendron flavens (Sw.) Griseb.

538. On *Guarea Swartzii* DC. $\Delta = 1.27$, $P = 15.3$
March 18. $\Delta = 0.90$, $P = 10.8$ $+ 0.37$, $+ 4.5$

528. On *Guarea Swartzii* DC. $\Delta = 1.34$, $P = 16.2$
March 18. $\Delta = 1.02$, $P = 12.3$ $+ 0.32$, $+ 3.9$

II. Phthirusa lepidobotrys (Griseb.) Eichl.

104. On *Dodonaea jamaicensis* DC. $\Delta = 1.24$, $P = 14.9$
Feb. 7. $\Delta = 1.09$, $P = 13.1$ $+ 0.15$, $+ 1.8$

510. On *Viburnum villosum* Sw. $\Delta = 1.36$, $P = 16.4$
March 18. $\Delta = 1.15$, $P = 13.9$ $+ 0.21$, $+ 2.5$

292.	On *Duranta repens* L.	$\Delta = 1.39$, $P = 16.7$
	Feb. 28. $\Delta = 1.29$, $P = 15.5$	$+0.10$, $+1.2$
280.	On *Hedyosmum nutans* Sw.	$\Delta = 1.42$, $P = 17.1$
	Feb. 26. $\Delta = 0.73$, $P = 8.8$	$+0.69$, $+8.3$
109.	On *Baccharis scoparia* (L.) Sw. Old leaves,	$\Delta = 1.45$, $P = 17.4$
	Feb. 7, Host not taken. New leaves,	$\Delta = 1.16$, $P = 13.9$
265.	On *Dodonaea jamaicensis* DC.	$\Delta = 1.59$, $P = 19.1$
	Feb. 26. $\Delta = 1.41$, $P = 16.9$	$+0.18$, $+2.2$

III. Phthirusa parvifolia (Sw.) Eichl.

133.	On *Clethra occidentalis* (L.) Steud.	$\Delta = 1.15$, $P = 13.8$
	Feb. 9. $\Delta = 0.77$, $P = 9.3$	$+0.38$, $+4.5$
356.	On *Mecranium purpurascens* (Sw.) Triana	$\Delta = 1.16$, $P = 13.9$
	March 4. $\Delta = 0.77$, $P = 9.2$	$+0.39$, $+4.7$
203.	On *Vaccinium meridionale* Sw.	$\Delta = 1.18$, $P = 14.2$
	Feb. 18.[3] $\Delta = 1.25$, $P = 15.1$	$-.07$, -0.9
481.	On *Palicourea alpina* (Sw.) DC.	$\Delta = 1.23$, $P = 14.8$
	Feb. 16. $\Delta = 0.83$, $P = 10.0$	$+0.40$, $+4.8$
483.	On *Clethra occidentalis* (L.) Steud.	$\Delta = 1.28$, $P = 15.4$
	Feb. 16. $\Delta = 0.68$, $P = 8.2$	$+0.60$, $+7.2$
537.	On *Quercus* sp.	$\Delta = 1.30$, $P = 15.6$
	March 19. $\Delta = 1.10$, $P = 13.3$	$+0.20$, $+2.5$
364.	On *Ilex montana* (Sw.) Griseb.	$\Delta = 1.31$, $P = 15.8$
	Sap from host was insufficient for a determination.	
246.	On *Vaccinium meridionale* Sw.	$\Delta = 1.38$, $P = 16.7$
	Feb. 24. $\Delta = 1.34$, $P = 16.1$	$+0.04$, $+0.6$
484.	On *Vaccinium meridionale* Sw.	$\Delta = 1.39$, $P = 16.7$
	March 16.[4] $\Delta = 1.32$, $P = 15.9$	$+0.07$, $+0.8$
292.	On *Duranta repens* L.	$\Delta = 1.40$, $P = 16.8$
	Feb. 28. $\Delta = 1.29$, $P = 15.5$	$+0.11$, $+1.3$
204.	On *Baccharis scoparia* (L.) Sw.	$\Delta = 1.42$, $P = 17.1$
	Feb. 18. $\Delta = 1.15$, $P = 13.8$	$+0.27$, $+3.3$
196.	On *Dendrophthora gracilis* (Griseb.) Eichl.	$\Delta = 1.49$, $P = 17.9$
	Feb. 17. $\Delta = 1.26$, $P = 15.2$	$+0.23$, $+2.7$
247.	On *Baccharis scoparia* (L.) Sw.	$\Delta = 1.50$, $P = 18.0$
	Feb. 27. $\Delta = 1.27$, $P = 15.4$	$+0.23$, $+2.6$
265.	On *Dodonaea jamaicensis* DC.	$\Delta = 1.63$, $P = 19.6$
	Feb. 26. $\Delta = 1.41$, $P = 16.9$	$+0.22$, $+1.7$

IV. Phthirusa pauciflora (Sw.) Eichl.

110.	On *Baccharis scoparia* (L.) Sw.[5] Old leaves	$\Delta = 1.41$, $P = 17.0$
	New leaves	$\Delta = 1.31$, $P = 15.7$
	No determination made for the host.	

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[3] New leaves of the host gave: $\Delta = 0.93$, $P = 11.2$.

[4] New leaves of the host gave: $\Delta = 1.18$, $P = 14.2$.

[5] The identity of the parasite is in this case not quite certain.

508. On *Citharexylum caudatum* L. $\Delta = 1.59,\ P = 19.2$
March 18. $\Delta = 2.03,\ P = 24.4$ $-0.44,\ -5.2$
265. On *Dodonaea jamaicensis* DC. $\Delta = 1.71,\ P = 20.5$
Feb. 26. $\Delta = 1.41,\ P = 16.9$ $+0.30,\ +3.6$

V. DENDROPHTHORA CUPRESSOIDES (Macf.) Eichl.

130. On *Miconia quadrangularis* (Sw.) Naud. $\Delta = 0.99,\ P = 11.9$
Feb. 9. $\Delta = 0.87,\ P = 10.5$ $+0.12,\ +1.4$
226. On *Miconia quadrangularis* (Sw.) Naud. $\Delta = 1.04,\ P = 12.5$
Feb. 20. $\Delta = 0.94,\ P = 11.3$ $+0.10,\ +1.2$
491. On *Miconia quadrangularis* (Sw.) Naud. $\Delta = 1.08,\ P = 13.0$
March 16. $\Delta = 1.07,\ P = 12.9$ $+0.01,\ +0.1$
482. On *Miconia quadrangularis* (Sw.) Naud. $\Delta = 1.16,\ P = 14.0$
March 16. $\Delta = 0.98,\ P = 11.8$ $+0.18,\ +2.2$
403. On *Miconia quadrangularis* (Sw.) Naud. $\Delta = 1.17,\ P = 14.1$
March 9. $\Delta = 1.11,\ P = 13.4$ $+0.06,\ +0.7$
204. On *Baccharis scoparia* (L.) Sw. $\Delta = 1.19,\ P = 14.4$
Feb. 18. $\Delta = 1.15,\ P = 13.8$ $+0.04,\ +0.6$
122. On *Rapanea ferruginea* (R. & P.) Mez $\Delta = 1.25,\ P = 15.1$
Feb. 9.[6] $\Delta = 0.96,\ P = 11.6$ $+0.29,\ +3.5$
247. On *Baccharis scoparia* (L.) Sw. $\Delta = 1.26,\ P = 15.1$
Feb. 24. $\Delta = 1.28,\ P = 15.4$ $+ .02,\ +0.3$
486. On *Rapanea ferruginea* (R. & P.) Mez $\Delta = 1.45,\ P = 17.4$
March 16. $\Delta = 1.07,\ P = 12.9$ $+0.38,\ +4.5$

VI. DENDROPHTHORA GRACILIS (Griseb.) Eichl.

123. On *Eugenia virgultosa* (Sw.) DC? $\Delta = 0.91,\ P = 10.9$
Feb. 9. $\Delta = 0.72,\ P = 8.7$ $+0.19,\ +2.2$
180. On *Eroteum theoides* Sw. $\Delta = 1.10,\ P = 13.2$
Feb. 14.[7] $\Delta = 1.03,\ P = 12.4$ $+0.07,\ +0.8$
481. On *Palicourea alpina* (Sw.) DC. $\Delta = 1.12,\ P = 13.5$
March 13. $\Delta = 0.83,\ P = 10.0$ $+0.29,\ +3.5$
447. On *Vaccinium meridionale* Sw. $\Delta = 1.19,\ P = 14.3$
March 13. $\Delta = 1.36,\ P = 16.3$ $- .17,\ -2.0$
374. On *Eroteum theoides* Sw. $\Delta = 1.24,\ P = 14.9$
March 6. $\Delta = 1.20,\ P = 14.5$ $+0.04,\ +0.4$
284. On *Eroteum theoides* Sw. $\Delta = 1.25,\ P = 15.1$
Feb. 28. $\Delta = 1.15,\ P = 13.9$ $+0.10,\ +1.2$
196. On *Cyrilla racemiflora* L. $\Delta = 1.26,\ P = 15.2$
Feb. 17. $\Delta = 1.18,\ P = 14.2$ $+0.08,\ +1.0$
484. On *Vaccinium meridionale* Sw. $\Delta = 1.34,\ P = 16.1$
March 16.[8] $\Delta = 1.32,\ P = 15.9$ $+0.02,\ +0.2$

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[6] Young leaves of the host gave: $\Delta = 0.89,\ P = 10.7$.

[7] For new leaves: $\Delta = 1.30,\ P = 15.6$.

[8] For new leaves of host: $\Delta = 1.18,\ P = 14.2$.

VII. DENDROPHTHORA OPUNTIOIDES (L.) Eichl.

512. On *Dendropanax arboreum* (L.) Dec. & Pl.	$\Delta = 1.13$, $P = 13.6$
March 18. $\Delta = 1.10$, $P = 13.3$	$+0.03$, $+0.3$
153. *Dendropanax arborum* (L.) Dec. & Pl.	$\Delta = 1.16$, $P = 14.0$
Feb. 11. $\Delta = 1.11$, $P = 13.3$	$+0.05$, $+0.7$
509. On *Oreopanax capitatum* (Jacq.) Dec. & Pl.	$\Delta = 1.17$, $P = 14.0$
March 18.[9] $\Delta = 1.53$, $P = 18.3$	-0.36, -9.8

As a basis of comparison for the individual determinations for the hosts, the average freezing point lowering and osmotic pressure based on all the determinations made, not merely those from plants on which parasites were found, are given in the accompanying table.

MEAN OSMOTIC PRESSURE IN SAP OF HOSTS

Species	Determinations	Mean Δ	Mean P
Baccharis scoparia	3	1.177	14.17
Citharexylum caudatum	4	1.950	23.43
Clethra occidentalis	2	0.725	8.75
Cyrilla racemiflora	1	1.180	14.20
Dendropanax arboreum	2	1.105	13.30
Dodonaea jamaicensis	3	1.183	14.23
Duranta repens	5	1.288	15.50
Eroteum theoides	4	1.143	13.78
Eugenia virgultosa	1	0.720	8.70
Guarea Swartzii	7	0.894	10.77
Hedyosmum nutans	1	0.730	8.80
Miconia quadrangularis	8	0.988	11.90
Mecranium purpurascens	2	0.770	9.25
Oreopanax capitatum	3	1.593	19.10
Palicourea alpina	6	0.675	8.12
Quercus	1	1.100	13.30
Rapanea ferruginea	4	1.078	12.98
Vaccinium meridionale	6	1.292	15.55
Viburnum villosum	1	1.150	13.90

A cursory glance over the differences shows at once that in the great majority of the cases the freezing point lowering of the parasite is distinctly greater than that of the host.

This result may be most emphatically brought out by a graphical representation of the constants in diagrams 1 and 2. In diagram 1 the amount of depression below the freezing point of pure water for both parasite (solid dots) and host (circles) is shown on the scale to the left. Here the determinations are arranged in order according to the freezing point lowering of the parasite. Diagram 2 is quite comparable except that in it the data are arranged according to the freezing point lowering of the juices of the leaves of the host plant.

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[9] The young leaves of the host gave: $\Delta = 1.14$, $P = 13.8$.

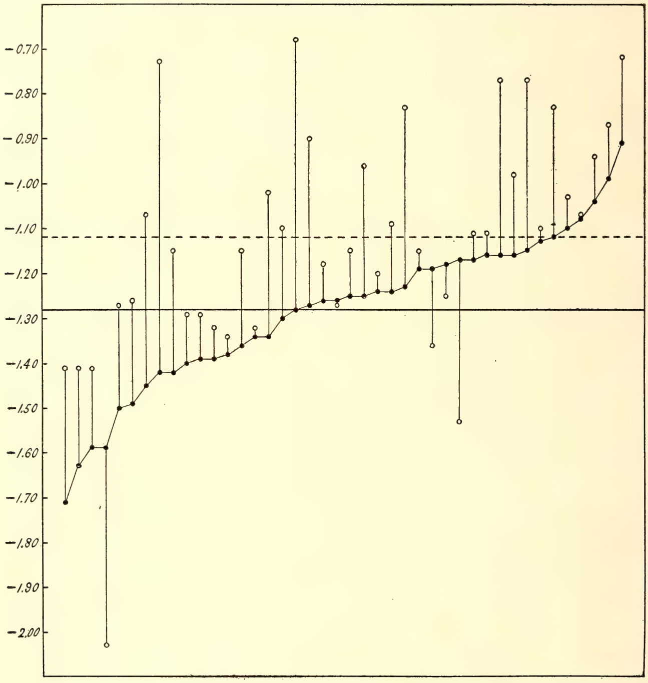

Diagram 1. Freezing point of extracted sap of parasite (solid dots) and of host (circles). The determinations are arranged in order of magnitude of the values obtained from the parasite. The length of the lines connecting the dots and circles indicate on the scale to the left the amount of the difference between the freezing point of the sap of the two organisms. The mean value for the parasites is shown by the solid bar, that for the hosts by the broken line.

The differences between parasite and host are indicated by the lengths of the bars connecting the circles and solid dots.

With but five exceptions, of which one is trivial in magnitude, the parasites show distinctly greater freezing point lowering than their hosts. In other words, their tissue fluids are characterized by higher osmotic pressure.

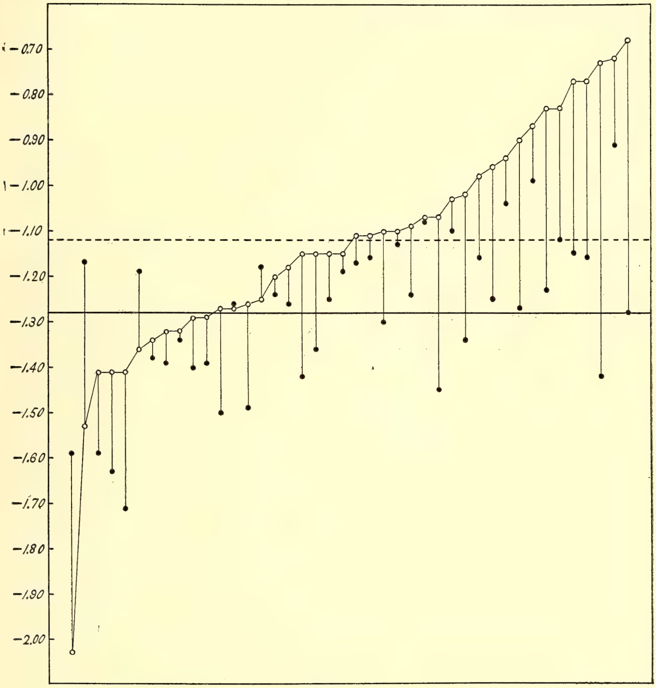

DIAGRAM 2. Determinations of freezing point of extracted sap of Loranthaceae and hosts, arranged in order according to the values for the latter. Compare explanation of Diagram 1.

Having recourse to averages for more exact comparison of the seven species of parasites with their hosts[10] we find:[11]

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[10] The means are based upon the parasites which are accompanied by host determinations only, not upon all the freezings for the sap of the loranths. Thus the means for host and parasite are quite comparable.

[11] The bars indicate that the values of Δ and P are averages.

Phoradendron flavens, 2 determinations

For parasite . . . . $\bar{\Delta} = 1.305$, $\bar{P} = 15.75$

For host . . . . $\bar{\Delta} = 0.960$, $\bar{P} = 11.55$

Difference . . . . $+0.345$ $+4.20$

Phthirusa lepidobotrys, 5 determinations

For parasite . . . . $\bar{\Delta} = 1.400$, $\bar{P} = 16.84$

For host . . . . $\bar{\Delta} = 1.134$, $\bar{P} = 13.64$

Difference . . . . $+0.266$ $+3.20$

Phthirusa parvifolia, 13 determinations

For parasite . . . . $\bar{\Delta} = 1.347$, $\bar{P} = 16.19$

For host . . . . $\bar{\Delta} = 1.110$, $\bar{P} = 13.38$

Difference . . . . $+0.237$ $+2.81$

Phthirusa pauciflora, 2 determinations

For parasite . . . . $\bar{\Delta} = 1.650$, $\bar{P} = 19.85$

For host . . . . $\bar{\Delta} = 1.720$, $\bar{P} = 20.65$

Difference . . . . -0.070 -0.80

Dendrophthora cupressoides, 9 determinations

For parasite . . . . $\bar{\Delta} = 1.177$, $\bar{P} = 14.16$

For host . . . . $\bar{\Delta} = 1.048$, $\bar{P} = 12.62$

Difference . . . . $+0.129$ $+1.54$

Dendrophthora gracilis, 8 determinations

For parasite . . . . $\bar{\Delta} = 1.176$, $\bar{P} = 14.15$

For host . . . . $\bar{\Delta} = 1.098$, $\bar{P} = 13.24$

Difference . . . . $+0.078$ $+0.91$

Dendrophthora opuntioides, 3 determinations.

For parasite . . . . $\bar{\Delta} = 1.153$, $\bar{P} = 13.86$

For host . . . . $\bar{\Delta} = 1.246$, $\bar{P} = 14.96$

Difference . . . . -0.093 -1.10

The first point to be brought out by these averages is that the osmotic pressure of the tissue fluids of the leafless species is distinctly lower than that of the juices extracted from the leaf-bearing forms. The mean depressions in the former are 1.153°, 1.176° and 1.177°, as compared with 1.305°, 1.347°, 1.400°, and 1.650° in the latter.

The second is the emphasis laid upon the higher osmotic pressure of the fluids of the parasite as compared with those of the host.

In five of the seven comparisons the mean osmotic pressure of the juices of the parasite is higher than that of the leaf tissue of its hosts. The two exceptions are represented by only 2 and 3 determinations each. They cannot therefore be considered important, but are included for the sake of completeness merely. It is interesting to note that numerically they are smaller than the positive differences.

With numbers so small as those involved in the series of determinations for the individual species, it is idle to calculate probable errors. This has, however, been done for the whole series.[12]

The results are:

For parasites . . . . $\overline{\Delta} = 1.282 \pm .018$, $\overline{P} = 14.43$
For hosts . . . . $\overline{\Delta} = 1.129 \pm .026$, $\overline{P} = 13.59$
$+ 0.153 \pm .032$ $+ 1.84$

The difference is over four and one half times as large as its probable error.

In the diagrams and the averages we have included every pair of determinations available for the Jamaican series to avoid any possible criticism concerning the selection of data. Some of the cases in which the leaves of the host are recorded as exhibiting a higher osmotic pressure than those of the parasite are perhaps capable of explanation. Consider these cases in detail.

The exceptions to the rule of the higher osmotic pressure of the sap of the parasite are the following:

203. *Phthirusa parvifolia* . . . . $\Delta = 1.181$
on *Vaccinium meridionale* . . . . $\Delta = 1.252$
0.071

247. *Dendrophthora cupressoides* . . . . $\Delta = 1.258$
on *Baccharis scoparia* . . . . $\Delta = 1.276$
0.018

447. *Dendrophthora gracilis* . . . . $\Delta = 1.19$
on *Vaccinium meridionale* . . . . $\Delta = 1.355$
0.164

508. *Phthirusa pauciflora* . . . . $\Delta = 1.593$
on *Citharexylum caudatum* . . . . $\Delta = 2.027$
0.434

509. *Dendrophthora opuntioides* . . . . $\Delta = 1.165$
on *Oreopanax capitatum* . . . . $\Delta = 1.525$
0.360

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[12] Statistically the process of combination of diverse species of parasites and of the various species of hosts seems quite legitimate, since the heterogeneity will tend to *increase* the magnitude of the calculated probable error.

In considering these exceptions it is important to note that the differences between parasite and host observed in this series as a whole are not large. The average differences for 42 determinations, regarding signs, is 0.154° while for the 37 cases in which the osmotic pressure of the parasite is greater than that of the host the average difference is only 0.203°.

Working as we did under many difficulties the greatest exactness in the constants cannot be expected. In at least one of these exceptions the difference between the freezing point lowering of the two organisms is so small that it may be due to purely experimental errors. Thus one would hardly assert that such a difference as 0.018° for Dendrophthora on Baccharis furnishes any valid proof that the osmotic pressure of the sap of these parasites is really lower than that of the leaves of their hosts.

With regards to these exceptions the following biological factors should be pointed out.

In the case of Collection 203, the value for the parasite is among the three smallest out of the 14 made for *Phthirusa parvifolia*. It may, therefore, be actually too small. The host tree had apparently been injured by fire recently. Old and young leaves of the host were taken. The young leaves gave only $\Delta = 0.931$. Thus the parasite ($\Delta = 1.181$) shows a concentration distinctly higher than do the young leaves of the host which are drawing their water in competition with the old ones.

The difference between the freezing point of *D. cupressoides* and its host, *B. scoparia*, is, as has already been pointed out, so slight that it might easily be due to an error in the determination of the freezing points alone. The constant for Baccharis is the highest secured for that species, which because of the hardness of its tissues presents some difficulty in the extraction of sap. Another point will be mentioned below.

Thus of the 42 comparisons there seem to be only three, that is those numbered 447, 508 and 509 in which the evidence of a superior osmotic pressure of the leaves of the host can be given any weight at all. These three cases are particularly interesting.

Note first of all that two of these three exceptions are leafless and rather thick stemmed shrubs, *Dendrophthora gracilis* and *D. opuntioides*. It is quite possible, as explained above, that in these cases there has been included a considerable amount of juice from woody tissue not at all comparable with the leaves of the host.[13]

Note too that while the juices of *D. opuntioides* gave a freezing point lowering of 0.360° less than that of the old leaves of Oreopanax, they gave a depression slightly greater than that of young Oreopanax leaves from the same tree, *i. e.*, $\Delta = 1.165$ as compared with $\Delta = 1.144$. The older leaves of Oreopanax gave a juice which was too gelatinous to filter. Whether or not this has influenced the accuracy of the determination of the freezing point of the host leaves we cannot assert.

There remains but one further exception, *Phthirusa paucifolia* on *Citharexylum caudatum.* At first sight the Δ of the host seems suspiciously high, but the three other determinations for this species made on trees from which parasites were not secured give $\Delta = 1.772$, $\Delta = 1.945$, $\Delta = 2.048$ with an average for the four of $\overline{\Delta} = 1.950$. There can, therefore, be no legitimate assumption that the constant for the host is too high. There seems, indeed, no valid objection to this exception to the rule of a higher osmotic pressure of the juices of the parasite.

In its bearing upon the problem of the relative osmotic pressure of the solutions in the tissues of the two organisms the following cases of two or more species of parasites occurring on the same host plant are of great interest.[14]

The cases are arranged by the hosts.

204.	*Baccharis scoparia*	$\Delta = 1.15$,	$P = 13.8$
	Phthirusa parvifolia	$\Delta = 1.42$,	$P = 17.1$
	Dendrophthora cupressoides	$\Delta = 1.19$,	$P = 14.4$
247.	*Baccharis scoparia*	$\Delta = 1.28$,	$P = 15.4$
	Phthirusa parvifolia	$\Delta = 1.50$,	$P = 18.0$
	Dendrophthora cupressoides	$\Delta = 1.26$,	$P = 15.1$
265.	*Dodonea jamaicensis*	$\Delta = 1.41$,	$P = 16.9$
	Phthirusa parvifolia	$\Delta = 1.63$,	$P = 19.6$
	Phthirusa paucifolia	$\Delta = 1.71$,	$P = 20.5$
	Phthirusa lepidobotrys	$\Delta = 1.59$,	$P = 19.1$
292.	*Durantia repens*	$\Delta = 1.29$,	$P = 15.5$
	Phthirusa parvifolia	$\Delta = 1.40$,	$P = 16.8$
	Phthirusa lepidobotrys	$\Delta = 1.39$,	$P = 16.7$

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[13] This is also true of *D. cupressoides* on Baccharis discussed in a preceding paragraph.

[14] In collecting samples of this kind it was often impossible to secure ample materials of both of the parasites from the same shrub or tree. In such cases great care was taken to obtain materials of both parasites from the same individual trees and to compound the sample of the host plant from these trees in order to avoid all possibility of having the comparability of the samples jeopardized.

481. *Palicouria alpina*.................................. $\Delta = 0.83$, $P = 10.0$
Phthirusa parvifolia............................ $\Delta = 1.23$, $P = 14.8$
Dendrophthora gracilis.......................... $\Delta = 1.12$, $P = 13.5$
484. *Vaccinium meridionale*............................. $\Delta = 1.32$, $P = 15.9$
Phthirusa parvifolia............................ $\Delta = 1.39$, $P = 16.7$
Dendrophthora gracilis.......................... $\Delta = 1.34$, $P = 19.1$

In all, the osmotic pressure of the juices of each of the two or three parasites is higher than that of the host, although they may differ sensibly between themselves.

One of the most interesting cases is that of a broad-leaved loranth, *Phthirusa parvifolia* parasitic upon a leafless member of the same family, *Dendrophthora gracilis* which is in turn parasitic upon a tree of *Cyrilla racemiflora* about twenty feet in height (Coll. 196).

The sap properties stand in the following relationship.

Cyrilla racemiflora...................................... $\Delta = 1.18$, $P = 14.2$
Dendrophthora gracilis.................................. $\Delta = 1.26$, $P = 15.2$
(on *Cyrilla racemiflora*)
Phthirusa parvifolia..................................... $\Delta = 1.49$, $P = 17.9$
(on *Dendrophthora gracilis*)

Osmotic pressure increases from host to the primary parasite and from the primary parasite to the secondary one.

Note also that the observed secondary parasitism is *Phthirusa parvifolia* with an average depression of 1.35° upon *Dendrophthora gracilis* with an average depression of 1.18°.

Whether the reciprocal case of secondary parasitization ever occurs could only be determined by more extensive field observation.

IV. Discussion of Results

The demonstration of the lower osmotic pressure of the tissue fluids of the leafless as compared with the leafy species of Loranthaceae requires no special discussion at the present time. The validity of this conclusion, based primarily upon averages, although also evident in the general trend of the individual constants, is evidenced for by certain favorable combinations of parasitism.

In four cases (Col. 204, 247, 481, 484) it was possible to secure determinations from a leafless and a leafy form upon the same host. As shown by the table above, the osmotic pressure of the leaves is higher in each case than that of the stems of the associated leafless parasite.

Note also that in the instance of secondary parasitism noted above, the primary parasite is leafless (Dendrophthora) and the secondary parasite (Phthirusa) leafy.

The problem of the relative magnitudes of the osmotic pressures of the fluids of parasites and host merits a short theoretical discussion. Let P_h be the observed pressure of the solution h in the tissues of the host, and P_p the observed pressure of the solution p in the tissue of the parasite. Then at first thought

$$P_p > P_h$$

would seem to be the necessary condition for the absorption of water from the host by the parasite. It is as a matter of fact the relationship almost always, but apparently not invariably, found. The view that it is the essential condition is of course quite fallacious, since p is not drawing water directly from h but the two are obtaining their water and its solutes from a common source, say t, the solution in the tracheae, with osmotic pressure P_t, which must be assumed to be less than that of either the solutions in the leaves of the host or the tissues of the parasites.

Now nothing is known concerning the concentration of the solutions in the xylem of the species upon which our determinations were based, but *a priori*, there should be no question in the minds of botanists that the osmotic pressure of the sap surrounding the haustoria of the parasite is distinctly lower than that of the leaf cells. Those who desire authority in support of this view may turn to the classic statement of Sachs that the water in the tracheae is an exceedingly dilute solution of nutritive salts which may be compared at once to ordinary drinking water.

Fortunately direct evidences are available for trees of temperate regions. Dixon and Atkins (1914, 1915) have given the following values for sap centrifuged from the wood in comparison with those obtained from the leaves after treatment with liquid air.

For *Acer pseudoplatanus*, in August
from tracheae of root . . . . $\Delta = 0.070$
from tracheae of branch . . . . $\Delta = 0.049$
from tissue of leaf . . . . $\Delta = 1.207$
For *Populus alba*, in August
from tracheae of root . . . . $\Delta = 0.072$
from tracheae of stem . . . . $\Delta = 0.047$
from tissue of leaf . . . . $\Delta = 1.487$

Clearly the values found for the leaves are enormously greater than those for the sap from the non-living conducting tissue of the stem. Further determinations for the wood of stem and root in *Acer pseudoplatanus*, *Cotoneaster frigida*, *Fagus silvatica*, *Ilex aquifolium*, *Populus alba* and *Salix babylonica* indicate that while the concentration of the fluids centrifuged from these stems varies rather widely during the year, concentrations comparable to those of the foliage leaf are never found. Indeed, the observed depression of such fluids is only a fraction of that prevailing in the leaf tissue of the typical arborescent plant.

Thus if the trunks of the ligneous hosts from which our parasites were taken present no higher concentrations in their fluids than do those of the trees studied by Dixon and Atkins,[15] there would seem to be no reasonable question of the adequacy of the osmotic pressures demonstrated in both the leaves of the host and the tissues of the parasite for any rôle which may be logically assigned it in maintaining a flow of water from the wood cells to the chlorophyll bearing tissues of both host and parasite.

Thus the condition

$$P_h > P_t, \quad P_p > P_t$$

where h represents the leaves and t the conducting system of the trunk of the host, may be reasonably assumed to obtain universally in the case of shrubby parasites on the aerial parts of the ligneous hosts.

Returning to the question of the relative values of P_h and P_p it is now evident that these merely draw from t in competition with each other. Were the supply of t inadequate then $P_p > P_h$ would be a necessary condition for the development of the parasite, but if the supply of t is not limited it seems theoretically quite possible for a parasite to flourish on a host which has leaves of a higher osmotic pressure drawing water from the vascular system in competition with it.

In such competition h would draw water from t until the osmotic pressure of the cell came into equilibrium with the tension of the surrounding cell wall. The rate of absorption would then be limited by this tension, and p would be free from the competition of h so long as the volume of t was sufficient for both. In just such a region as the Blue Mountains of Jamaica, with its heavy and well distributed precipitation, one would expect the water brought up from the soil to be continually adequate.

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[15] Incidentally it may be pointed out that if the conclusion reached by Dixon and Atkins, that sugars (monosaccharides, disaccharides or both) are found at all times in the tracheae of trees, be found to apply generally to ligneous plants, new light is thrown upon the much discussed question of the degree of parasitism in the Loranthaceae. Even if they draw their substance entirely from the xylem they can hardly be regarded as merely water and mineral parasites, provided the tracheae contain throughout the year sugars supplied from the cortex by way of the medullary rays and the wood parenchyma, as urged by Dixon and Atkins. The discussion of this point falls quite outside the scope of the present paper.

There now seems ample comparative evidence for the soundness of the reasoning here involved. Dixon and Atkins in a series of studies (1912, 1912*a*, 1912*b*, 1915*a*) have shown by very exact work on *Ilex aquifolium*, *Hedera Helix* and *Syringa vulgaris* that the osmotic pressure of the young leaves is generally lower than that of the older ones. We have in our own unpublished data constants for a wide range of species from rain forest, desert and mesophytic habitats. These show that in general lower osmotic pressure characterizes the sap of the young leaves. Nevertheless it is quite obvious that these young leaves are drawing water in competition with the old ones.

It is important to note that in the cases in which determinations were based upon the tissue fluids of young leaves as well as upon those of the old leaves of the host, the osmotic pressure of the juices of the parasite has always been found to be higher than that of the young leaves.

Most convincing evidence of the same kind is furnished by our studies of prolification of the fruit in Passiflora (Harris, Gortner and Lawrence, 1915). In this remarkable abnormality a whorl of incompletely fused carpels is formed inside the trimerous or tetramerous ovary wall. Extensive observations have shown that for electrolytes, measured by electrical conductivity, and for both electrolytes and non-electrolytes, measured by specific gravity and by freezing point lowering, the concentration of the fluids of the abnormal structure is almost invariably lower than that of the normal wall, in competition with which it must draw its solutions from the conducting system of the plant.

V. RECAPITULATION

In this paper, which is one of a series on various physiological, ecological and phytogeographical problems involving the investigation of the physico-chemical properties of vegetable saps, we present data on the osmotic pressure of the tissue fluids of tropical mistletoes parasitic on various ligneous hosts.

Osmotic pressure has in every case been calculated from the depression of the freezing point of saps extracted by pressure from previously frozen tissues. The conclusions are based upon 42 pairs of determinations distributed among seven species belonging to three genera of Loranthaceae parasitic on 19 species of host. All determinations here published were made on materials collected in the neighborhood of Cinchona, in the Blue Mountains of Jamaica.

These studies demonstrate with reasonable certainty:

(*a*) That the sap extracted from the tissue of the green stems of the leafless species belonging to the genus Dendrophthora are characterized by a lower osmotic pressure than that obtained from the leaf tissue of the leafy species of Phoradendron and Phthirusa. These are of the order 1.170° and 1.350° freezing point lowering or about 14.2 and 16.2 atmospheres pressure respectively.

(*b*) That the osmotic pressure of the sap extracted from the chlorophyll bearing tissues of the parasite is almost always, but apparently not invariably, greater than that expressed from the mature leaves of the host.

While higher osmotic pressure of the sap of the parasite is a general condition, we have shown that it is not a *necessary* condition for at least the temporary success of the parasite. The parasite should be able to draw from the relatively dilute solutions in the stem in competition with organs of actually higher osmotic pressure, except at periods when the supply of soil moisture is limited, just as young leaves are able to draw water in competition with old leaves of higher osmotic pressure.

In conclusion we have to express our hearty thanks to Professor Bower and the other members of the British Association Committee having in charge the Tropical Laboratory at Cinchona for its protracted use, to Dr. MacDougal, director of the Department of Botanical Research, for material assistance in field operations, and to Dr. Britton, for the opportunity of having the materials upon which this and other unpublished physiological studies on Jamaican plants were based critically examined at the Herbarium of the New York Botanical Garden. To Mr. Percy Wilson of the Garden we are indebted for the painstaking care with which this work was carried out. To Mr. Wm. Harris, F.L.S., Superintendent of Public Gardens and Plantations, Jamaica, we are indebted for many courtesies which contributed

not merely to the success of our work but to the pleasure of our stay in Jamaica.

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FOUR-LOBED SPORE MOTHER CELLS IN CATHARINEA

Charles E. Allen

In examining some preparations of sporophytes of Catharinea, made from fixed material, it was noticed that the spore mother cells were plainly four-lobed. The preparations were laid aside until living material could be studied. This was done during the past summer, with a resulting confirmation of the former observations. Spore mother cells having the four-lobed form have now been found in developing sporophytes collected and fixed in September, 1907, in the neighborhood of Madison; and in living sporophytes collected during August, 1915, at three different localities in and near Madison, and at one locality near Devil's Lake, about thirty miles distant from Madison.

As appears from figure 1, *A–D*, there are rather striking differences in size between the spore mother cells of plants collected in different localities, although the mother cells borne in a single capsule differ little in size, and, so far as my observations have gone, there is no great variation in this respect as between different plants growing in the same clump. Along with these differences in size of spore mother cells between plants of different localities go other well-marked differences in such characters as size of plant, size of leaf, number of lamellae on the upper surfaces of the leaves, and number of spines on the lower surfaces. While doubtless some of these characters are influenced by external conditions, it seems not unlikely that the plants in question may represent distinct races, all of which, however, seem to fall within the limits of *C. angustata* Brid., as at present defined; although, since this species intergrades with *C. undulata* Web. and Mohr, it is possible that some of the forms observed should be referred to the latter species.

The spore mother cells shown in figure 1 were drawn (with the aid of the camera lucida) immediately after being pressed out of the living capsules. The lobing of the cells, though evident, is much shallower than in such representatives of the Jungermanniales as

Pellia[1] or Symphyogyna.[2] An occasional cell (as in figure 2, *C*) might be described as tetrahedral rather than lobed; the one shown in figure 2, *C*, however, could be seen in other optical sections than the one drawn to be really somewhat more distinctly lobed than this figure would suggest. The shapes of these cells in Catharinea are not dissimilar to those of the spore mother cells of Fossombronia.[3] Indeed, among the Jungermanniales, in which (if the Sphaerocarpaceae and Riellaceae be excluded) the lobing of the spore mother cells seems to be a universal character, the depth of the

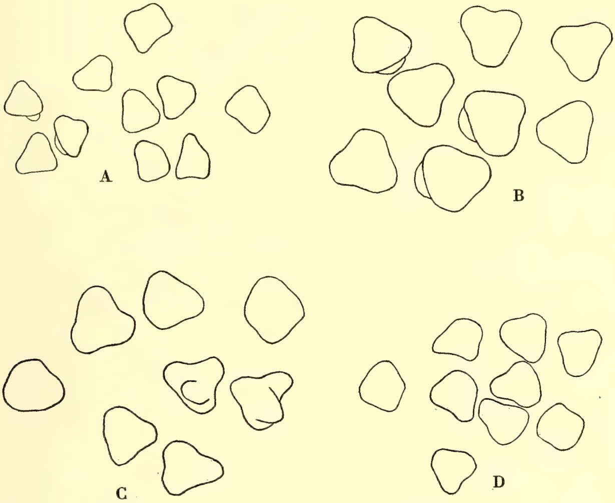

FIG. 1. Groups of spore mother cells from four capsules of *Catharinea angustata* From living material.

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[1] Farmer, J. B. On spore-formation and nuclear division in Hepaticae. Annals of Botany **9**: 469–523. 1895.

[2] McCormick, Florence A. A study of *Symphyogyna aspera.* Bot. Gaz. **58**: 401–418. 1914.

[3] Farmer, J. B. *Loc. cit.*

Humphrey, H. B. The development of *Fossombronia longiseta* Aust. Annals of Botany **20**: 83–108. 1906.

lobing varies greatly. This is shown, for example, by the differences in this respect between two species so closely related as *Blyttia* (*Pallavicinia*) *decipiens*[4] and *B. Lyellii*.[5]

Figure 2 shows stages in the division of the spore mother cell, as seen in sections of fixed material. At the earliest stage here shown (figure 2, *A*), the cell contains four plastids which are located respectively in the four lobes of the cell. At this time the plastids seem to have the form of curved plates; later they become more rounded but by no means spherical; the apparent differences in size and shape

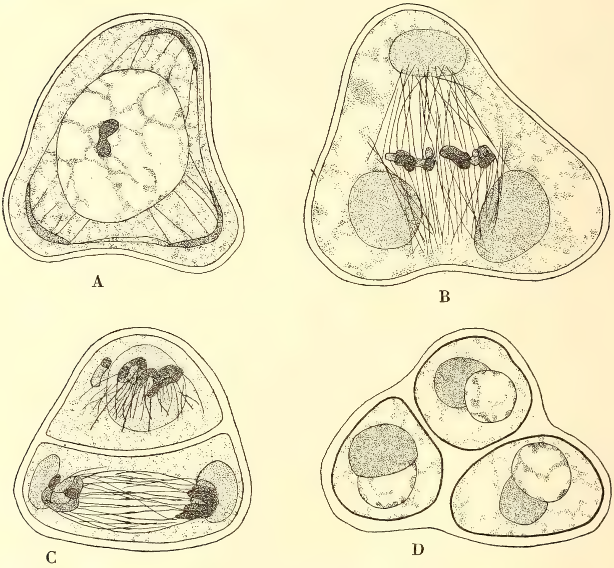

FIG. 2. Stages in the division of the spore mother cell and spore formation in *Catharinea angustata*. From fixed material.

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[4] Farmer, J. B. Studies in Hepaticae: On *Pallavicinia decipiens* Mitten. Annals of Botany **8**: 35–52. 1894.

[5] Moore, A. C. Sporogenesis in Pallavicinia. Bot. Gaz. **40**: 81–95. 1905.

between the different plastids shown in figure 2, *B–D*, are at least in part due to the fact that these somewhat flattened bodies are seen from different angles.

The fibers that appear in figure 2, *A*, running from each plastid toward the nucleus, seem to be the beginnings of a spindle which in its origin is quadripolar. The mature spindle is bipolar (figure 2, *B*). Each blunt pole lies between two plastids; the upper pole of the spindle shown in figure 2, *B*, is not directed toward a plastid, as the figure might suggest, but lies at a higher focus and between this plastid and another one, not present in the section from which the figure was drawn. Both the first (heterotypic) and the second (homoeotypic) nuclear divisions are followed by the formation of cell plates and of partition walls. Figure 2, *C*, shows the wall that divides the spore mother cell after the first nuclear division, as well as the beginning of the formation of a cell plate on one of the second division spindles. As this figure also shows, the spindles of the second division are so oriented that each spindle pole is directed toward, and in contact with, one of the four plastids. Consequently, each of the daughter nuclei resulting from this division lies in contact with a plastid (figure 2, *D*). The formation of partition walls after the second mitosis completes the division of the spore mother cell into four spores, each of which contains a nucleus and a plastid and corresponds substantially (except for the nucleus) to one of the lobes of the mother cell.

After cell division has been completed, the original wall of the mother cell and the partition walls separating the spores gradually soften. The spores round up somewhat, and each forms an independent wall, at first quite thin, about itself and inside the softening substance of the older walls (figure 2, *D*). Finally the dissolution of the old walls reaches the stage at which the spores become free.

This is, I think, the first observed case of the occurrence of four-lobed spore mother cells in a bryophyte not a member of the Jungermanniales. It remains to be seen to what extent this character appears among the Bryales. Such studies of sporogenesis as have been made in members of the latter order seem to indicate that in general the mother cells are not lobed. The taking on of a lobed form is of interest because it anticipates the division of the cell at a time when there is no evidence either of a preparation for division on the part of the nucleus or of the development of the spindle mechanism. Another anticipatory step is seen in the early division of a single plastid into four, a process which, known for some time[6] to occur in Anthoceros, is also, to judge from the work of Sapěhin[7] and Melin,[8] of common occurrence among the Musci.

[6] Davis, B. M. The spore-mother-cell of Anthoceros. Bot. Gaz. **28**: 89–109. 1899.

[7] Sapěhin, A. A. Über das Verhalten der Plastiden im sporogenen Gewebe. Bericht. Deutsch. Bot. Ges. **29**: 491–496. 1911.

[8] Melin, E. Die Sporogenese von *Sphagnum squarrosum* Pers. Nebst einigen Bemerkungen über das Antheridium von *Sphagnum acutifolium* Ehrh. Svensk Bot. Tidsk. **9**: 261–293. 1915.

THE WANDERING TAPETAL NUCLEI OF ARISAEMA

F. L. Pickett

The fact that in certain of the higher plants the walls of the tapetal cells break down and allow the nuclei and cytoplasm to "wander" among the developing microspores has been repeatedly noted. In the recent paper on wandering tapetal nuclei, Juel (1915) has carefully reviewed the findings of earlier workers and made important criticisms upon their reports. The great advance in technical methods has made it advisable to go carefully over the ground covered by Strasburger

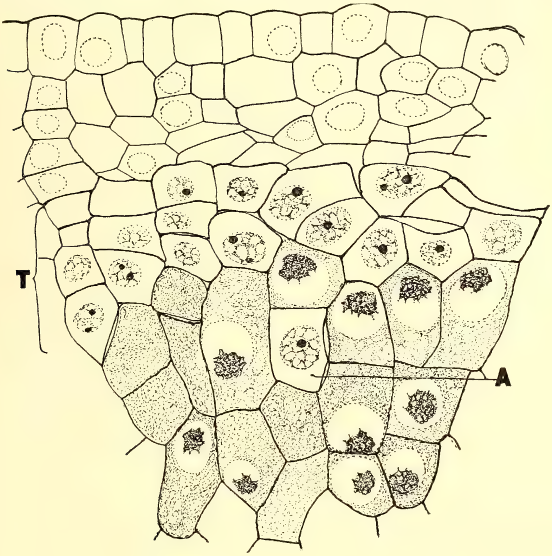


FIG. 1. Section of anther showing pollen mother-cell nuclei in synapsis and the tapetal cells, *T*, with the first indication of separation. A single sterile cell is shown at *A* surrounded by sporogenous cells. × 280.

and others. This Juel has done quite thoroughly, and in doing so has added much to our knowledge of the behavior of the tapetal cells. The wide range of plants in which the wandering nuclei have been found seems to indicate that much more time may well be spent in observation along this line.

Within the past year the writer (Pickett, 1915) reported the occurrence of wandering tapetal nuclei in *Arisaema triphyllum* (L.) Schott. The work of Juel which appeared while the writer's paper was in the publisher's hands, has made it seem advisable to go over the *Arisaema triphyllum* material again and make a fuller report. At the same time a study of *Arisaema Dracontium* (L.) Schott. has

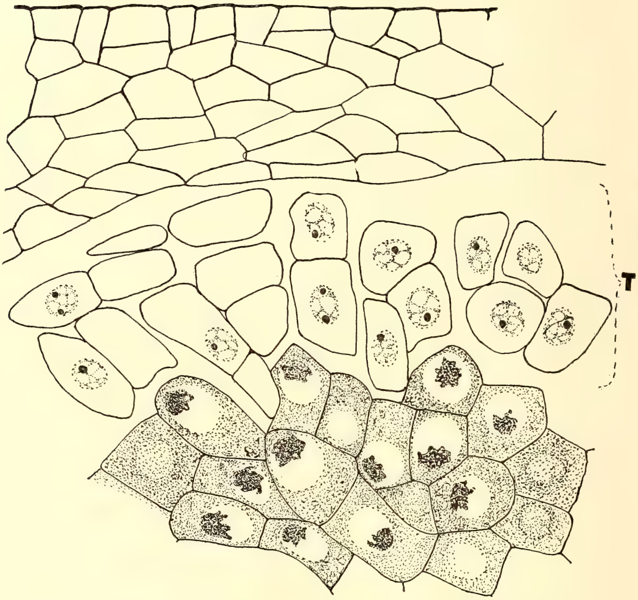


FIG. 2. A section of an anther in which the tapetal cells, *T*, have almost entirely separated. × 280.

been made, and the reports concerning the two species are here presented together.

Material for the study was collected in Indiana in 1914 and 1915. That most used was fixed in chrom-acetic acid (chromic acid 1 gm.,

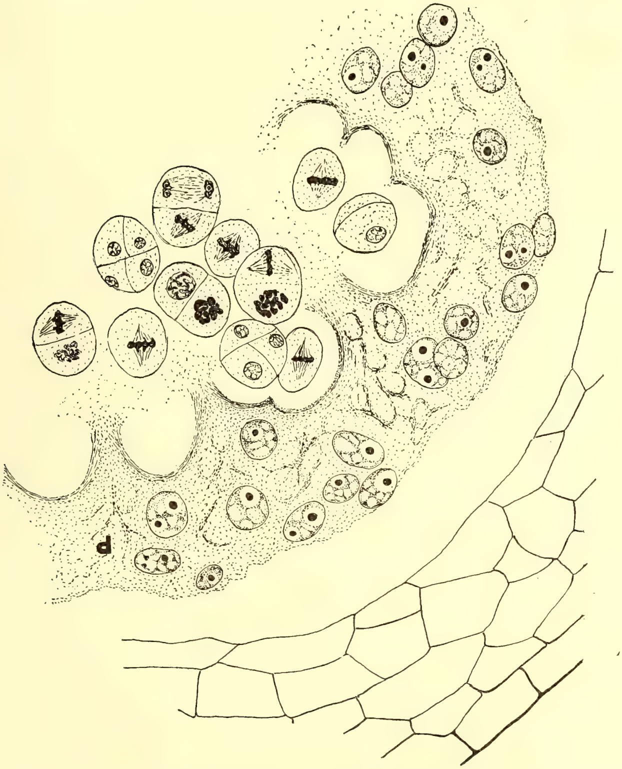


FIG. 3. Section of anther showing the periplasm, *P*, composed of the mixed masses of protoplasm from the tapetal cells and carrying their nuclei. The protoplasmic mass has not yet spread through the spaces between the dividing pollen mother-cells nor filled the anther cavity. × 280.

acetic acid 1 cc., water 99 cc.). Other fixing agents were used for check material but without giving any differences with respect to the points under consideration. After the usual infiltration and imbedding in paraffin the material was sectioned 5–8 μ in thickness, and finally stained in Haidenhain's iron-alum haematoxylin, in a similar stain in which a 1/10 percent aqueous solution of brazilin replaced the usual ½ percent solution of haematoxylin, and in various modifications of the triple stain, safranin-gentian violet-orange G. The best differentiation was obtained by the use of clove oil solutions of both the gentian violet and orange G as suggested in the Annals of Botany (**29**: 471–472. 1915). By the use of this stain it was always easy to differentiate the tapetal nuclei in the periplasm and to make out the smallest details of their structure. The brazilin was found of value also in the study of details of nuclear structure. A clove oil solution of "Licht-Grün" used after the triple stain above mentioned or after the Haidenhain's haematoxylin was found useful in distinguishing

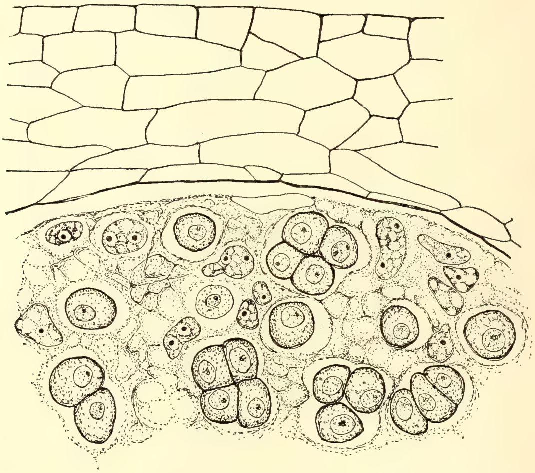


FIG. 4. A portion of an anther cavity filled with the periplasm. The tapetal nuclei have begun to change form and wander among the developing spores. × 280.

between the cells of the anther wall, whose cellulose stained readily and strongly, and the tapetal cells whose walls were but lightly stained if at all.

The conditions and phenomena connected with the tapetum in the two species of Arisaema were found to be almost identical, so this report is made with primary reference to *A. triphyllum*. The drawings have all been made from preparations of material of that species. These have been carefully checked and the development of the microspores has been carefully compared with material of *A. Dracontium*. The photomicrographs in plate XX were taken from preparations of the latter species.

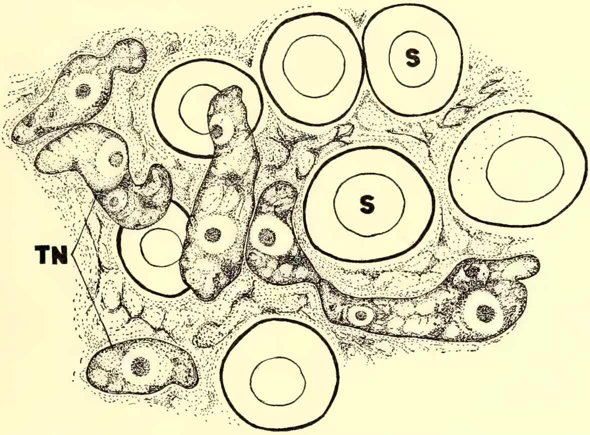


FIG. 5. A small portion of an anther cavity showing various forms of tapetal nuclei, *TN*, among developing pollen spores, *S*. × 1400.

A section of an anther, made just before the beginning of the heterotypic division of the pollen mother-cell nuclei, shows its wall composed of a single layer of epidermal cells, and, in its thinnest part, a single row of sterile cells which later elongate radially and become thick walled. Within these two tissues there is a third, the tapetum, from two to four cells thick. The drawings have been made from portions of sections which show clearly the relation of the tapetum to the sporogenous tissue regardless of the part of the anther wall included. The tapetal cells are clearly differentiated from those of the anther wall by their denser cytoplasm and larger nuclei. After the last vegetative division of the sporogenous cells, the tapetal cells may be distinguished, although less easily, from the pollen mother-cells by their more vacuolate cytoplasm and by peculiarities of nuclear structure. Nuclei of pollen mother-cells show the finely divided chromatin and delicate linin network typical of such nuclei, while the tapetal nuclei show larger and more irregular masses of chromatin (text figure 7). With the appearance of the close synaptic ball in the nucleus of the pollen mother-cell, the typical resting nucleus serves to mark each tapetal cell even when such a cell is, as occasionally found, entirely surrounded by sporogenous cells (figure 1*A*).

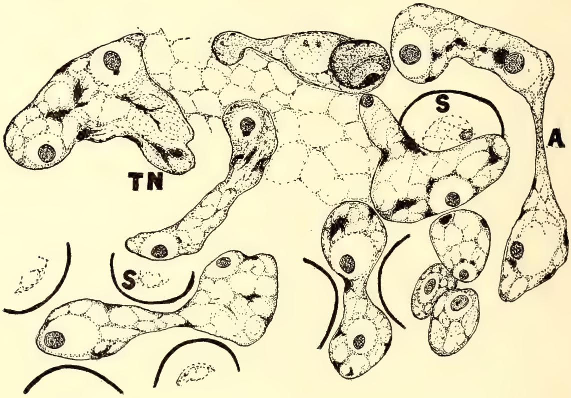


FIG. 6. A group of almost mature pollen spores, *S*, and tapetal nuclei, *TN*, the latter showing the chromatin masses characteristic of late stages. The smaller and more regular nuclei represent those with lessened activity. The nucleus at *A* suggests simple division. These nuclei are from different parts of one anther section; only enough portions of pollen spores are shown to indicate relative size and position. × 1400.

When the tissues are first differentiated the tapetal cells are closely packed together and show distinct walls and well vacuolated cytoplasm. As the sporangial cavity enlarges through the extension of its wall cells the tapetal cells show a tendency to pull apart and round up (text figures 1 and 2). This separation seems to indicate that normal extension of these cells has ceased and the continued enlargement of the cavity has left them without external pressure, for measurements of the cells at this time compared with similar measurements made before their separation shows no appreciable shrinkage. At this time the middle lamella has entirely disappeared and in a short time the cells, rounded up and floating in the cell sap of the sporangial cavity, lose through hydrolization and solution all of their remaining cell walls. When the heterotypic division is completed the mass of pollen mother-cells is completely surrounded by the tapetal protoplasm and free floating nuclei (text figure 3). This mass of protoplasm immediately streams in between the dividing cells, surrounding each one and filling the entire sporangial cavity (text figure 4; plate XX, figure 1). From this time until the spores separate, the pollen mother-cells and the forming tetrads are within large, vacuole-like spaces in the tapetal protoplasm. The spreading of the protoplasm through the sporangial cavity is accompanied or immediately followed by a migration of the tapetal nuclei and their approximately equal distribution throughout the cavity. All this time the tapetal protoplasm or the periplasm of Hannig (1911) has shown a highly vacuolate structure.

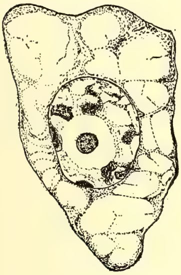


FIG. 7.

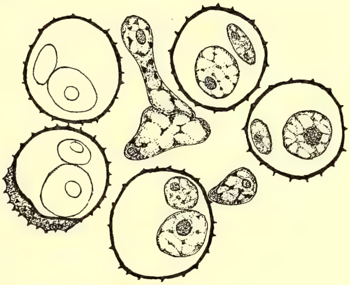


FIG. 8.

FIG. 7. A normal tapetal cell before the disappearance of the wall, showing the highly vacuolate cytoplasm, and characteristic nuclear structure. × 1400.

FIG. 8. Almost mature pollen grains and a few persistent tapetal nuclei. × 1000.

It now becomes even more highly vacuolate, the spaces about the spores disappear and an actual contact between the protoplasm and the spore walls is evident in places.

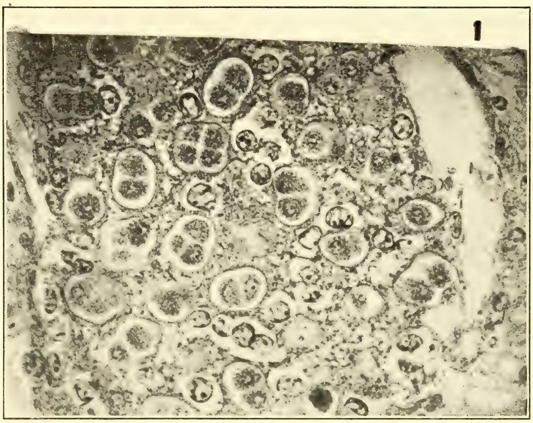
Up to the time of the general migration through the sporangial cavity, the tapetal nuclei largely retain their original form. With the movement of the protoplasm they slip between the tetrads and at the same time become slightly larger and considerably distorted in form. A marked change in nuclear structure appears. Each nucleus shows many large, irregular vacuoles and often two or more nucleoli each surrounded by a large, definite vacuole (text figure 5). They continue to increase slightly in size and show more widely varying forms, up to the time of exine formation by the pollen spores. It has been as yet impossible to demonstrate active movement on the part of these nuclei; but it is difficult to find any other explanation of the appearance of their peculiar forms so long after their first migration through the cavity. There has been found no indication of mitosis in these nuclei, and only occasional forms which seem to indicate increase in number by simple division (text figure 6, *A*) have been observed.

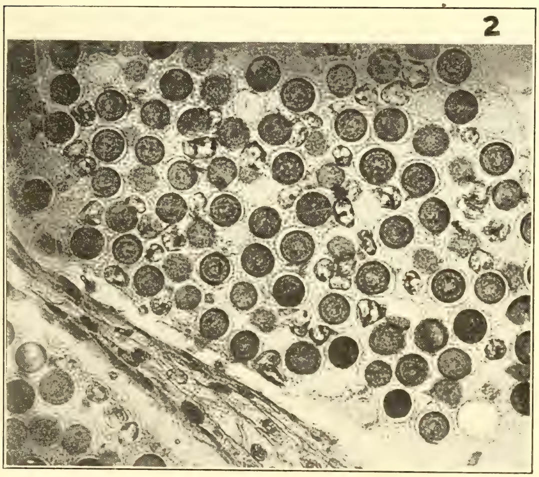
With the maturing of the pollen grains the protoplasm surrounding them becomes less dense, with finer strands separating the more sharply marked vacuoles (text figure 6), and finally disappears. Along with this change in the protoplasm the wandering nuclei shrink, show a decrease in general density and a gathering of the chromatin into small, almost opaque masses (text figure 6). In many cases the nuclei become more regular in form before finally shrivelling up with the loss of water when the pollen grains mature. In a few cases they retain much of their characteristic appearance until quite late (text figure 8), although most of them have lost all indications of activity before the stage of maturity indicated in this figure.

Summary

The study reported at this time shows that in the development of the microsporangia of *Arisaema triphyllum* (L.) Schott. and *A. Dracontium* (L.) Schott. the tapetum is early differentiated by peculiarities of cell wall, nuclear and cytoplasmic structure.

The walls of the tapetal cells entirely disappear, allowing the several protoplasmic masses to form a periplasm spreading through the sporangial cavity.





PICKETT: WANDERING TAPETAL NUCLEI OF ARISAEMA.

The tapetal nuclei for a considerable period show peculiarities of structure, and take on amoeboid forms suggestive of active migration among the developing pollen spores.

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LITERATURE

Inasmuch as the material presented above is intended merely to report observations of fact without going into any discussion of their possible bearing upon development or possible relationship of the species studied, only such literature has been cited as may help to make clear the findings reported. For a more complete bibliography see Juel's work.

Hannig. Über die Bedeutung der Periplasmodien. III. Kritische Untersuchungen über das Vorkommen und die Bedeutung von Tapeten und Periplasmodien. Flora, **102**: 335–382. 1911.

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Pickett. A Contribution to Our Knowledge of *Arisaema triphyllum*. Mem. Torrey Club. **16**: 1–55. 1915.

EXPLANATION OF PLATE XX.

FIG. 1. A section showing the periplasm with tapetal nuclei among the spore tetrads. × 375.

FIG. 2. A similar view from a section of an older anther, showing tapetal nuclei in periplasm among spores with well developed exine. × 375.

TWO TYPES OF VARIABLE PUBESCENCE ON PLANTS

P. L. Ricker

Pubescence on plants has long been made use of by systematic botanists as a character upon which species are frequently based, wholly or in part. In instances in which this combined with other constant characters it may be of value, but observations in the field and herbarium covering a long period of years have led to the belief that in a much larger proportion of cases than is suspected pubescence has been a delusion and a snare to the systematist. To most systematists doubtless a hair is a hair unless these differ noticeably in structure, size, or abundance, and little or no thought is given to the function of hairs or their possible lack of diagnostic value.

It is probable that all pubescence on plants, and perhaps glaucescence, may be divided into two classes, viz.: functioning and nonfunctioning. As an example of the latter class may be cited the well-known and extremely variable *Paspalum floridanum* Michx., in almost any patch of which one may find at least six forms as follows:

1. Plants with only the basal leaves and sheaths pubescent.
2. Plants with the basal leaves and sheaths and the sheaths of all cauline leaves pubescent.
3. Plants with all leaves and sheaths pubescent.
4. Plants with all leaves and sheaths and the stem pubescent as far as the rachis.
5. Plants pubescent to the tip of the inflorescence.
6. The more glabrous forms are in some cases glaucous.

In this species at least, the pubescence may be the result of a mutation and does not vary throughout the season. At least it does not appear to have any definite function. What would be the results of a study of the progeny of each form is uncertain, but probably the majority of the offspring would be like the parent with a scattering of all of the other forms.

In plants with functioning pubescence are found greater possibilities of deception to the systematic botanist than in the former. It has long been observed that the leaves of certain plants lose their pubescence with age and that leaves of other plants have one form of pubescence in the spring succeeded by a somewhat different kind of pubescence at maturity, but I do not know of attention having been called to any having glabrous leaves, and leaves with one and some with two forms of pubescence on a single individual at the same time. During the latter part of 1913 there was received for study, through the Office of Foreign Seed and Plant Introduction and Distribution, about thirty sheets of Chinese chestnuts (Castanea) collected by Mr. Frank N. Meyer in the province of Chi-li. They were accompanied by a lot of burs which were collected on a different date than any of the specimens, which were from two or three localities, so there was no reason to suspect that all belonged to the same species, especially as the leaves presented noticeable variations in size, pubescence, and dentation. Tentatively these were divided into three species and an unsuccessful attempt made to locate them among those previously described; but the absence of inflorescence and definite knowledge as to which of the specimens, if any, the burs were to be associated with, prevented any positive identifications. As the seed with this material was sent for propagation, with the expectation that it would prove resistant to the Chestnut Blight, Messrs. J. Franklin Collins and R. Kent Beattie, who were investigating this disease, went over the material and verified my tentative conclusions. Later, photographs of types from European herbaria and all of the Chinese Castanea from the Arnold Arboretum having been obtained, the material was again gone over with Messrs. Collins and Beattie without very satisfactory results, but with the firm conviction that three or more species were represented.

Learning from Mr. Collins that two trees propagated from the seed received were growing upon Mr. David Fairchild's place at North Chevy Chase, Md., where they had been inoculated the previous season, a joint inspection of the trees was arranged and all of the available type photographs and herbarium material taken along for comparison, with the following results.

1. Practically every form and size of leaf and variation in pubescence and dentation represented in the photographs of type material of *Castanea mollissima* Blume from the Paris and Leyden herbaria, and in the herbarium material from northern China, were found on the two trees which were grown from the same lot of seed. Furthermore the seeds from which the two trees grew probably were produced on the same tree in China.

2. All the leaves on the south side of the trees receiving the greatest amount of direct sunlight were densely velvety-pubescent beneath with stellate hairs thickly interspersed with long jointed hairs, the amount of pubescence gradually decreasing towards the base of the branch as the lower leaves received an increasing amount of shade from those above, the lowest leaves being practically glabrous.

3. Leaves on the north side of the tree receiving a minimum amount of sunshine showed practically no pubescence except at the tips of the youngest unfolding leaves which had a few of the long jointed hairs and an occasional stellate hair. Leaves on the east and west sides showed intermediate amounts of pubescence.

4. Leaves on short branches arising from the very base of the tree, and in deep shade at all times due to its low dense branching habit, were practically glabrous, or with a very few short jointed hairs along the midrib beneath. The leaves were also much smaller and of somewhat different shape and proportions from those on any other parts of the tree.

From the above facts, which have been further verified by an examination of plants growing at the Arnold Arboretum and by statements by Mr. Meyer since his return from China, it can only be concluded that *Castanea mollissima* is an extremely variable species and that in this species at least pubescence functions primarily as a protection to the young growing leaves from excessive transpiration and is of no diagnostic value. There has been no opportunity to study living material in the fall, but from herbarium material it may be concluded that most of the pubescence is lost by that time. It can not be argued that leaves on the tree without much pubescence are older than those that are densely pubescent and have lost their pubescence through age, because the youngest growing leaves, which would be the ones to be pubescent if any, are practically glabrous on the north side of the tree if protected at all. On the other hand those on the south side are densely pubescent in every case, unless growing on short branches near the trunk and entirely shaded by leaves on the longer branches above.

These facts serve to show the necessity for a more careful study of growing plants and the ease with which a systematist working with herbarium material alone may be deceived as to the diagnostic value of certain characters. In many instances living wild plants at points a few hundred miles apart which are apparently distinct will be found to have several intergradations in the intermediate territory. Doubtless all manuals of botany contain many overlooked cases of this kind, and specialists are even accused of describing two species from different parts of the same tree. While the unnecessary multiplication of species is to be regretted, it is manifestly impossible for but few systematists to compare critically many related species from growing material and the difficulties of the authors of manuals are far greater. It is, however, urged that all botanists give more careful attention to observations of this character in the field and that a greater amount of discriminating collecting be done with respect to variations occurring upon individual plants. A very large number of descriptions in the manuals of botany make no mention of the color of the flowers or of conspicuous root characters. As the descriptions in these manuals must of necessity be largely drawn from dried and often inadequate, or discolored specimens, but little improvement in the descriptions can be expected until herbaria are supplied with field labels giving the necessary data.

Bureau of Plant Industry,
Washington, D. C.

THE SEAWEEDS OF HAWAII

VAUGHAN MACCAUGHEY

"The Hawaiians, like the Japanese, are fond of almost all the products of the sea, and, like them, prize the seaweed very highly for food. Ancient Hawaiians probably seldom ate a meal without some kind of *limu* or seaweed. . . ."—Reed.

The Hawaiian Archipelago is about two thousand miles long, and lies athwart the center of the north Pacific ocean. Vast and deep marine expanses isolate it from both the American and the Asiatic continents. The waters that wash its thousand miles of diversified coast line are not tropic, but moderately warm—sub-tropic. Hence its coral reefs are small as compared with the great reefs of the South Pacific. Its seaweeds do not exhibit the profusion of species nor the luxuriance of form and color that characterize the oceanic flora of strictly tropical regions. Moreover, the giant kelps and laminarias that dominate the long cold coasts of northwestern America are conspicuously absent from Hawaiian shores and reefs.

The Hawaiian Islands rise abruptly from abyssal depths. Many of the shore-lines are exceedingly precipitous. Certain geologists have compared the islands to the summits of a row of obelisks. The area of shallow water is much more circumscribed than is generally supposed. The tracts possessing life-conditions favorable for the development of marine algae are distinctly limited in area and localized in distribution. The lower, older islands to the northwest have the largest reefs and shallows. These areas become progressively smaller toward the high, young, volcanic islands of the southeast. The island of Hawaii, youngest member of the archipelago, is distinguished by an almost entire absence of low beachlands, reefs, lagoons, or shallows. These topographic conditions have profoundly influenced the algal and other marine life of the Hawaiian group.

An hour's cruise in an outrigger canoe over a typical fringing reef is sufficient to reveal the five main zones of the algal flora. The shallow in-shore waters, with a bottom of coral sand or mud, sustain a number of the quiet water forms. Partially exposed rock masses of coral or lava are scattered here and there along the beach and in the shallow water. These rocks are horizontally banded with hydroid and algal colonies. Further out there are numerous "pockets" or cup-like depressions in the lagoon floor. These vary in size from a meter in depth and diameter to large pools five or ten meters in depth and diameter. These pockets are easily marked by the darker tint of their water. In them live a variety of organisms that prefer these shadowy havens to the exposure of the shallows or the surf-smitten outer reef.

The next zone is one of deeper water, where wading is no longer possible. The sunny, transparent water is three to ten meters deep, but becomes shallower as the edge of the reef is approached. On the outer rim pound eternally the long rollers of the Pacific, with iridescent spray haze and the deep-toned roar of the surf. Beyond this rim the reef drops abruptly in the abyssal waters of the Pacific. These five zones—shallows, rocks, pools, rim, and outer face of reef—each have distinctive combinations of ecologic factors, and each supports a distinctive flora and fauna.

Several hundred species of marine algae, exclusive of microscopic and unicellular forms, have been collected in Hawaiian waters. These represent a wide range of genera—green, blue-green, brown and red groups—and of habitats. Many species are as yet undescribed and await taxonomic investigation. Among the better represented genera are: Codium, Padina, Halimeda, Dictyota, Turbinaria, Ectocarpus, Enteromorpha, Bryopsis, Caulerpa, Laurencia, Gelidium, Griffithsia, Haliseris, Sargassum, Hypnea, Ulva, Polysiphonia, Porphyra, Hydrodictyon, Gracilaria and Ceramium.

Of great significance as reef builders are the coralline algae or nullipores. There are a number of species that inhabit the shallow waters, forming beautiful purple and lavender incrustations; others occur at considerable depths. The Hawaiian reefs, like those of other regions, undoubtedly owe a considerable proportion of their structure to plant depositions.

Many of the marine algae occupied places of prominence in the dietary of the ancient Hawaiians, and still constitute a staple in the native food. The villages, like those of other parts of Polynesia, were almost universally situated on or near the seashore. The ancient Hawaiians were preeminently a maritime people. They were intimately familiar with the products of the sea. A large part of the population was more or less habitually engaged in fishing. The deep sea fishing was naturally the work of the men, as it involved protracted labor and a certain degree of hazard. The reef-fishing was enjoyed alike by men, women, and children. From the lagoons and shallower waters was obtained a wide variety of marine edibles—fish of many kinds, crabs, crayfish and shrimps, molluscs, holothurians, sea urchins, octopi, etc. The edible seaweeds, *limu*, formed an important element in this native exploitation of the shallow waters.

Doubtless no primitive people made more extensive use of marine products than did the Polynesian in his sea-girt island world. About seventy-five species were used for food, and for these the ancient Hawaiian had specific names. It is of distinct interest to note that the Japanese, whose commercialized seaweed industries have been so widely described and studied, have a small number of edible species as compared with the Hawaiian. In Smith's monumental report on the Seaweed Industries of Japan (U. S. Bureau of Fisheries), only thirty-five species are reported as used for food, and ten others for the manufacture of gelatine, glue, iodin, etc. Thus the Hawaiian marine flora, although relatively poor in quantity, is rich in economic species.

The generic name for all kinds of algae was *limu*; to this was added in true Linnaean spirit, one or more descriptive terms. This is a truly noteworthy instance of parallelism in the evolution of nomenclature. The botanical nomenclature familiar to every learned Hawaiian of the old regime was essentially identical with the binomial system evolved by Caucasian scientists. The following examples of the old names for seaweeds will serve to elucidate this point:

Limu ele-ele ("the black or dark limu"), *Enteromorpha flexuosa.*
Limu lo-loa ("the long or slender limu"), *Gelidium* sp.
Limu koele ("the dry or hard limu"), *Gymnogongrus* sp.
Limu wawae-iole ("the mouse-foot limu"), *Codium muelleri.*
Limu huna ("the concealed or hidden limu"), *Hypnea* sp.
Limu koko ("the red limu"), *Asparagopsis sanfordiana.*

The *limu* was collected in various ways, depending upon the nature of the habitat. Some kinds (Sargassum, Gracilaria) drift ashore in tolerable abundance, and were easily gathered. Others growing in the quiet shore waters (Ulva, Entermorpha, Chondria, etc.) were readily collected by the older women and the children. Others, with stout stems and hold-fasts, occupied the black lava rocks in rough water. They were exposed to continuous surf-pounding and could only be collected by skilful swimmers, the men and younger women. A sharp stone or chisel was used to separate these *limu* from their rocky substratum. Gelidium and Porphyra belong to this class. A fourth class (Gymnogongrus, Dictyota, etc.) inhabited the outer edge of the reef, where the heavy rollers break. These were usually gathered by the men in outrigger canoes. A few species (e. g, *Porphyra leucostica*) occur only in certain restricted localities, or during brief seasons. These were either pre-empted by the chiefs as choice delicacies befitting nobility alone, or were consumed locally, and were not in general use among the people.

It is interesting to note that the Hawaiians anticipated by many centuries some of the recently advocated plans of limnologists for cultivating aquatic vegetation of economic value. A crude form of *limu* culture was practiced by the Hawaiian nobility in olden time. Rare and choice varieties were transplanted to the vicinity of the chief's beach home, where they were protected and easily available. Sometimes the fish ponds were thus used as alga-gardens. In these places the other algae were weeded out, and the semi-"cultivated" forms developed much more luxuriantly than they otherwise would have done. An example of one of these ancient royal *limu* gardens occurs near the beach residence of ex-Queen Liliuokalani, at Honolulu.

Since the coming of the white man the collecting of seaweeds has been greatly facilitated by the use of such simple aids as glass-bottomed "water-boxes," and sharpened iron rods. These have been generally adopted by the natives, who quickly perceived their utility. In ancient times the *limu* gatherer was compelled to rely on his unaided vision and a simple stone chisel.

After the *limu* was brought ashore the women took charge of it. They sorted the various kinds into separate piles, washed each in several changes of salt or fresh water. All sand, grit and other inedible matter was thoroughly removed. After cleansing the *limu* was salted and chopped or broken into small fragments. Certain species decayed rapidly if washed in fresh water; these were rinsed in salt water and eaten soon after preparation.

The *limu* was eaten raw, like a salad or relish. It was the universal accompaniment of the fish that formed an essential part of the native diet. Occasionally, when the supply of customary vegetable food (such as taro, sweet potatoes, or yams) fell short, as in times of war or famine, the *limu* was used as a substitute. It was then cooked in the underground oven, *imu*, with the meats.

A number of algae, both marine and fresh-water forms, were customarily treated to a native "ripening" process. This *limu* was soaked in fresh water for twenty-four hours or more, causing partial decomposition and the development of a strong odor. The filamentous algae common in mountain streams were considered to be particularly suitable for "ripening," and were generally used only after this peculiar treatment.

Limu was eaten in combination with many other foods, rather than as a food by itself. It corresponded to the salad, or to the ripe olives and salted almonds, of an American dinner. The finely-chopped *limu* was mixed with any one or with combinations of the following: raw-fish, squid, shrimps, limpets, crabs, sea-urchins, holothurians, *kukui* nuts, chili peppers. A favorite relish was made from the roasted kernels of the *kukui* nuts (*Aleurites moluccana*, or candle-nut), which were chopped fine and mixed with *limu* and salt. According to Miss Reed, who a number of years ago made a careful study of the edible seaweeds of Hawaii, "This will keep for months in glass jars, and is excellent with bread and butter or cold meats. It resembles Russian caviar in flavor. . . . The Hawaiian serve this with poi, raw or cooked fish, or roast meats."

Vegetable preparations of various kinds comprised a large part of the native pharmacopeia. The *limu*, although not an important item in the long list of Hawaiian medicines, were used in several ways. Certain filamentous species (Spirogyra, etc.) were used as poultices for sore eyes. A number of kinds were used as poultices for cuts, bruises, sores, and boils. An infusion of Centroceros was used as a cathartic, and *Hypnea nidifica* was similarly employed for stomach troubles.

The edible seaweeds were so extensively and so variously used in ancient Hawaii that it is difficult to make any accurate estimate of the quantities then consumed. In modern times, despite the great shrinkage of the native population, *limu* forms a staple article of merchandise at the fish markets. In Honolulu, the chief market, the annual sales amount to about five thousand pounds, selling at about $2,500. This comprises chiefly *limu kohu* (*Asparagopsis sanfordiana*), *limu ele-ele* (*Enteromorpha* spp.), and *limu o-olu* (*Chondria tenuissima*). Hawaii's preponderant Oriental population, that now makes over sixty percent of the total, uses large quantities of seaweed. The Japanese import annually about 165,000 pounds, almost wholly from Japan. The Chinese import about 90,000 pounds from their country.

With the passing of the Hawaiian much of the old *limu* lore will be lost. In time, as marine biological stations and facilities for research develop, it will be supplanted by the precise data of modern algology. Hawaii's seaweeds will always be an interesting component of the diversified marine life of this mid-Pacific archipelago.

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SPECIFIC ACTION OF BARIUM

W. J. V. Osterhout

In order to arrive at a satisfactory theory of living matter it is necessary to know the specific manner in which individual substances affect metabolism. Usually our attempts to arrive at this kind of knowledge are very unsatisfactory. When plants grow in water cultures we can say what elements are indispensable but as a rule we can not tell with certainty by inspecting a plant what particular element is lacking in the nutrient medium in which it grew. Similarly when plants are killed or injured by poisons we can seldom by inspection say exactly what particular agent produced the effect. It would seem however that precisely this sort of knowledge should be sought for on account of its theoretical and practical importance.

In the course of investigations in the action of salts the writer has found cases in which this kind of information is apparently obtainable. One of the most striking of these is observed when certain species of *Spirogyra* are subjected to the action of barium.

The *Spirogyra* used in most of these investigations was a large form of the *crassa* type. On placing this in .0001 M $BaCl_2$ there was a peculiar and very characteristic contraction of the chloroplasts in the center of the cell. In the neighborhood of the nucleus the chloroplasts contracted so strongly that they formed a very compact green mass, like a closely twisted rope. The diameter of this mass was about one fourth to one third of that of the cell. At the ends of the cell little or no contraction occurred. At the same time the wavy outline of the chloroplasts disappeared so that their edges became relatively even and smooth (this however is not a specific effect of barium).

An interesting feature of this process is that the protoplasm does not contract away from the cell wall but remains in place. It is

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therefore evident that this process is different from false plasmolysis, although false plasmolysis may occur later if the exposure be sufficiently prolonged.

None of the other salts examined produced the characteristic contraction at such dilutions. $SrCl_2$ produced it at a higher concentration (.001 *M* and higher) but $CaCl_2$ and $MgCl_2$ did not produce it even at concentrations which plasmolyzed; the same is true of $MnCl_2$, $CdCl_2$, $NiCl_2$, $CoCl_2$, NaCl, KCl and NH_4Cl. As long as we work with very dilute solutions the effect of barium appears to be specific.

Even if it should turn out that other salts can produce this effect (the action of trivalent kations was not investigated) the striking fact remains that calcium and magnesium, which are chemically closely related to barium, do not produce it at all.

It would appear that further investigation of this and similar cases is desirable in order to discover what constituents of protoplasm make this specific action possible.

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STUDIES ON EXOSMOSIS

S. C. Brooks

In experiments on permeability where the turgidity of plant cells or their osmotic pressure (as judged by that of a solution just concentrated enough to plasmolyze them) is used as a criterion, there is an important source of error, which is usually overlooked. This lies in the possibility that osmotically active substances may diffuse out of the cell.

It was noted as early as 1860 by Knop, in connection with water cultures, that plant organs bathed by distilled water may give off substances to it. This phenomenon, usually termed exosmosis, may take place when tissues which are not normally in contact with water are placed in contact with distilled water or dilute solutions. Thus Wächter (7), found that strips of onion bulb scale gave off sugars to distilled water, and that this exosmosis was hindered by 0.1 to 0.4 M solutions of potassium and sodium chlorides and potassium nitrate.

Recently True and Bartlett (4, 5, 6) have made a thorough investigation of the intake and outgo of salts from roots of field peas grown in distilled and river water, and in solutions (mostly 0.001 M) of various salts singly and in combinations. A somewhat similar investigation by Merrill (1, 2) included the study of the effects of salt concentrations as high as 0.1 M.[1]

In all these experiments solutions were used whose concentration was far below that necessary to cause plasmolysis, and the effects were observed after periods up to fifty days, at which time the pure salt effects might be obscured by the readjustments of the organism to its change in environment. They do not, therefore, enable us to distinguish the immediate effects of salts on the plasma membrane, nor do they help us to determine the possible rôle of exosmosis in experiments whose duration is a matter of a few hours at most, as is the case in most experiments in which the turgidity of cells or their recovery from plasmolysis is used as a criterion of their permeability.

---

[1] It is not known to what extent the results (in all the cases here cited) may be due to the death of superficial cells of the roots.

A great many conclusions as to the nature of protoplasm or its surface layer (the so-called "plasma membrane") have been drawn from experiments on plasmolysis. But since the alterations in turgidity or in degree of plasmolysis may not only be increased by the entry ("endosmosis") of osmotically active substances from the solution bathing the cell, but may also be decreased by outward diffusion of similar substances ("exosmosis"), the rate of which may be altered by the plasmolyzing agent, it seemed highly desirable to observe the effect on exosmosis immediately following the application of solutions isotonic with the cells of the material used.

A series of such experiments was conducted in which the exosmosis of electrolytes into distilled water from strips of peduncles of the dandelion (*Taraxacum officinale* Weber)[2] was determined immediately following a previous treatment with distilled water or with sodium, calcium, or cerium chlorides.

METHOD AND PRECAUTIONS

In these experiments the best grade of water distilled from glass was used; the salts were Baker's "analyzed" sodium chloride, Kahlbaum's calcium chloride, and Merck's "Reagent," cerium chloride. The solutions were made up with a maximum error of 0.5 percent. That this accuracy was sufficient will be seen from the fact that a change in the concentration of the $CaCl_2$ solution from 3 percent below to 3 percent above that isotonic with the sodium chloride solution used produced no appreciable difference in the results of the experiment. Solutions were considered to be isotonic with the cells when there was for a few seconds a barely perceptible decrease in the curvature, as observed by the use of a microscope, of freshly cut strips of peduncle on immersion in the solution. A detailed discussion of the method of determination, and of the accuracy and significance of this criterion will be presented in a subsequent paper.

A peduncle was cut into pieces 5 cm. in length, and these were cut longitudinally into as many strips as there were solutions to be investigated; one strip from each piece was placed in each solution. About fifteen or twenty pieces of peduncle were so used. Since relative results only were sought, the number of strips used was not important; it was only necessary to divide each piece accurately, so that the aggregate amount of material should be the same in the different solutions. The strips were protected from evaporation until all were cut; each lot was then placed in a test-tube, rinsed with distilled water which was allowed to drain off for thirty seconds, and the solutions were then poured in.

---

[2] The dandelions were grown in the greenhouse from wild plants dug up in the autumn. The plants were in the height of flowering when the material was used.

In these experiments three solutions were used: sodium chloride 0.22 *M*, calcium chloride 0.16 or 0.17 *M*, cerium chloride 0.050 *M*. In the control experiment distilled water took the place of a salt solution. After a period of from fifteen to twenty-five minutes these solutions were poured off and the material was rinsed three times with distilled water, the second change remaining in contact with the tissue two minutes. The last rinsing was allowed to drain off for thirty seconds, and then 13.0 cc. of distilled water placed in each test-tube. This amount was just sufficient to cover the strips of tissue, which were packed loosely in the bottom of the test tube. At the end of fifteen minutes the distilled water was poured off into a specially constructed U-tube designed to contain 13 cc. of solution, and its conductance determined. The solution was then returned to the material, and its conductance determined in a similar manner at suitable intervals.

Preliminary experiments indicated the possibility that some substance gathered on the electrodes, forming there a highly resistant layer. It was therefore decided to interpose parchment thimbles between the electrodes and the solution. These thimbles were kept in distilled water, and were placed (filled with distilled water) in the expanded ends of the U-tube just prior to each measurement. Figure 1 will show the arrangement of the U-tube, the bright platinum electrodes, and the solutions. It will be seen that the current traversed always the same amount of distilled water and the same length of column of solution. The distilled water in the parchment thimbles was not changed during a single set of three or four readings; it was possible at the end of a set to duplicate so closely the first reading taken, that it was evident that the diffusion of electrolytes into the distilled water in the thimbles introduced no appreciable error.

The largest source of error was that introduced by a certain amount of distilled water which it was impracticable to remove from the outside of the parchment thimbles before introducing them into the U-tube. The error thus introduced was not, however, sufficient to cause any significant variation in the results of the experiment. The

U-tube was placed in a constantly stirred water bath, whose temperature was determined to within 0.1° C., and the conductance determined by means of the ordinary arrangement of a slide-wire bridge, Nernst string inductorium, standard 1,000-ohm coil and telephone receiver. The error was less than 1 percent. The fluctuations in temperature were not great enough to justify the introduction of a temperature correction.

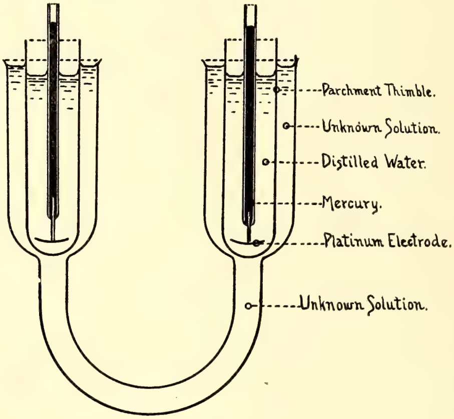

FIG. 1.

In the later experiments it was found that the parchment thimbles could be omitted, with a corresponding gain in the accuracy of the readings. The conductance was then determined between the two electrodes, now immersed directly in the solution. This fact prevents the direct comparison of readings taken by the different methods, but, as the comparative values remain unchanged, does not destroy the significance of the experiments.

It is obvious that the total increase in the conductance of the distilled water in contact with material which has been immersed in a salt solution will measure not only exosmosis from the protoplasm, but also diffusion from the intercellular material (*i. e.*, all non-protoplasmic material, including cell walls and intercellular spaces). This diffusion from intercellular material will change the conductance of the distilled water to a degree dependent on the concentration and molecular conductivity of the salt solution used, and will be absent in the control experiment, in which distilled water takes the place of a salt solution.

It is possible, however, to determine the duration of this diffusion, and by comparing the rate of change of conductance subsequent to its practical completion to gain an insight into the effect of salts on exosmosis from the protoplasm. Tissue which has been treated with distilled water for twenty-four hours has practically ceased to give off electrolytes to the distilled water. If such tissue be treated with salt solutions, as described above, it will be found that after thirty minutes the rate of change of conductance parallels that found for the same material treated with distilled water. This fact is apparent from the data in Table 1, and is represented graphically in Figure 2,

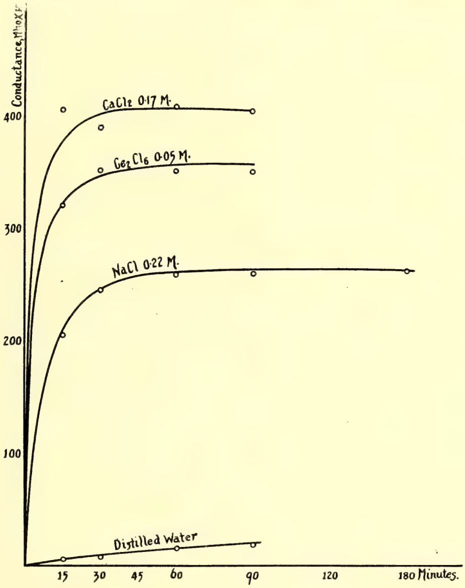

FIG. 2.

in which the ordinates represent conductance (in ohms) and the abscissæ the time which elapsed between placing the material in distilled water and the determination of the conductance.

Table I.

Diffusion of Salts from Tissue Leached in Distilled Water for a Period of Twenty-three Hours Previous to Treatment with the Salt Solutions

Time in Minutes After Transfer to Distilled Water	Conductance in Ohms × 10^7 of Distilled Water in Contact with Tissues Previously Exposed 25 Min. to:			
	NaCl 0.22*M*	$CaCl_2$ 0.17*M*	Ce_2Cl_6 0.05*M*	Distilled Water
15	205	405	320	5
30	245	389	351	7
60	258	407	350	15
90	259	403	350	18
180	261	—	—	—

In this experiment the differences of conductance are caused almost exclusively by the diffusion from the tissues of the salts with solutions of which they have been treated; the conductances are therefore closely proportional to the conductances of these solutions. The experiment shows that diffusion of the absorbed salt from the tissues is completed within thirty minutes, and the increase of conductance in the different solutions subsequent to this time may be attributed largely to exosmosis from the protoplasm.

RESULTS

If strips of dandelion peduncle be immersed (after momentary rinsing) in distilled water there is a steady exosmosis of electrolytes, and the rate of this exosmosis decreases gradually and without sudden change during the duration of the experiment, a matter of from six to eight hours. Since the protoplasm is normally in equilibrium with a more or less concentrated solution permeating the intercellular substance, the replacement of this solution by distilled water will necessarily lead to a disturbance of the equilibrium, and may, without causing any marked change in the normal permeability of the protoplasm, lead to an abnormal diffusion of substances from the cell, or, in other words, to an abnormal exosmosis. A previous treatment of the tissue with an isotonic solution of sodium chloride has the effect of accelerating this exosmosis, which, on the other hand, is inhibited by an isotonic solution of calcium chloride.

The first effect of cerium chloride is, like that of calcium, an inhibition of the exosmosis, but this effect is quickly reversed, and the rate of exosmosis becomes greater than that from any other of the lots of material. This is probably due to the not inconsiderable toxicity of the solution of cerium chloride, enough of which probably remained in the protoplasm to cause injury, with consequent greatly increased exosmosis. The effect of sodium chloride is only temporary, disappearing within one and one quarter hours after the removal of the tissue from the sodium chloride solution.

Table 2.

Effect of Salts on Exosmosis from Unleached Tissue

Time in Hours After Transfer to Distilled Water	Conductance in Ohms, $\times 10^7$, of Distilled Water in Contact with Tissues Previously Exposed 20 Min. to:			
	NaCl 0.22 *M*	$CaCl_2$ 0.17 *M*	Ce_2Cl_6 0.05 *M*	Distilled Water
0.50	28.0	44.5	24.0	8.0
1.25	41.0	49.5	27.0	16.0
3.00	48.0	51.5	40.0	26.0
4.57	51.0	53.0	45.5	31.0
5.90	51.5	53.3	47.0	32.0

The data are given in Table 2, and are graphically presented in Figure 3, in which the ordinates represent the total gain in conductance at intervals of time after the end of the period of thirty minutes which was allowed for diffusion of salts from the intercellular material.

A consideration of the fact that after the first thirty minutes the exosmosis from tissue which had been treated with calcium chloride was less than that from tissue which had not been in contact with any salt solution, shows that the substance causing the increase of conductance was not, or at least only to an extremely small extent, the salt used; the data therefor show that sodium salts increase, and calcium salts decrease the permeability of the protoplasm to substances other than themselves.

By analogy with the experiments of Osterhout (3) on *Laminaria* it should be possible to find some mixture of salts which, in a solution of the proper concentration, would leave the permeability of the dandelion protoplasm unaltered. Such a combination was found. It was not possible to determine exactly its optimum constitution; but a solution consisting of 80 parts sea water and 20 parts of a 0.52 *M* solution of calcium chloride, diluted to 21/52 of its original concentration, was found to present approximately the desired "normal" conditions for the protoplasm of the dandelion. In Table 3 will be found the constitution of this solution in gram molecules per liter, and

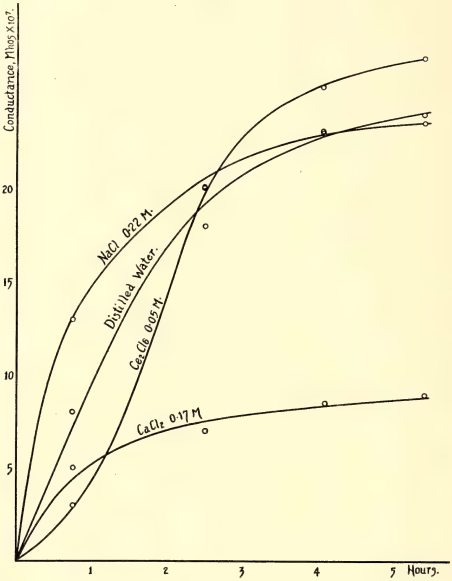

FIG. 3.

TABLE 3

Constitution of a Solution Favorable to the Protoplasm of Taraxacum officinale, and That of Sea Water at the Same Dilution

Salt	Sea Water		Solution	
	Gm. Mols per Liter	Percent of Total Gm. Mols	Gm. Mols per Liter	Percent of Total Gm. Mols
NaCl	0.2020	86.2	0.1608	68.4
$CaCl_2$	0.0046	2.0	0.0465	19.8
$MgCl_2$	0.0158	6.7	0.0158	6.7
$MgSo_4$	0.0077	3.3	0.0077	3.3
KCl	0.0046	1.9	0.0046	1.9
Total..........	0.2347	100.1	0.2354	100.1

the percentage of the total number of gram molecules present in the form of each of the constituent salts. The corresponding figures for sea water diluted to the same extent are given for comparison.

The effect of this solution on exosmosis from the protoplasm (after the first thirty minutes) does not differ appreciably from that of distilled water. The rate of exosmosis lies always intermediate between those for sodium and calcium chlorides, as will be seen from the data given in Table 4, and graphically presented in Figure 4.

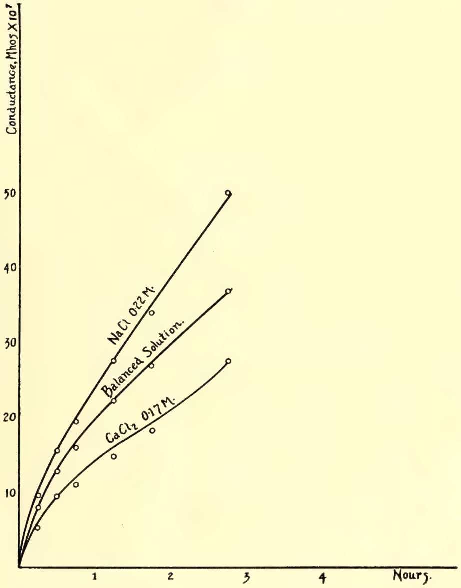

FIG. 4.

Results wholly analogous with those given above were secured after much shorter exposures to the salt solutions. Effects could be detected even after a four-minute exposure to a sodium chloride solution.

TABLE 4

Effect of Balanced and Pure Salt Solutions on Exosmosis from Unleached Tissue

Time in Hours After Transfer to Distilled Water	Conductance in Ohms, × 10^7, of Distilled Water in Contact with Tissues Previously Exposed 30 Min. to:		
	Balanced Solution	NaCl 0.22M	$CaCl_2$ 0.17M
0.75	55.0	78.5	73.5
1.00	63.0	88.0	78.7
1.25	67.8	94.0	83.0
1.50	71.0	98.0	84.5
2.00	77.8	106.0	88.3
2.50	81.8	112.3	91.7
3.50	91.7	128.4	100.1

SUMMARY

1. Sodium salts increase the rate of exosmosis of other electrolytes from the protoplasm of *Taraxacum officinale*.

2. Calcium salts decrease this rate.

3. A solution may be prepared consisting of a mixture of various salts in proportions such that, when used at a concentration isotonic with the protoplasm, it causes no appreciable alteration in the permeability of the plasma membrane of *Taraxacum officinale*.

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EFFECT OF ENVIRONMENTAL CONDITIONS UPON THE NUMBER OF LEAVES AND THE CHARACTER OF THE INFLORESCENCE OF TOBACCO PLANTS[1]

H. A. Allard

Introduction

Among tobacco growers it is a matter of common observation that the number of commercial leaves produced by tobacco plants varies considerably from season to season. It is commonly believed that drought and other conditions unfavorable to growth not only produce smaller sized leaves but also reduce the number of leaves. Since in actual field practice any count of the number of leaves is only approximately accurate, an investigation was made to determine the true number of leaves and the character of the inflorescence produced by tobacco plants under different conditions of environment.

Since the inflorescence of the tobacco plant is determinate or centrifugal in its mode of flowering there is no further production of internodes with the appearance of the terminal flower bud. The terminal blossom is the first to appear, followed by other blossoms arising from the nearest or inmost buds of the branches representing the terminal whorl. The lowest lateral branch of the inflorescence of the main stem is popularly termed the first bald sucker from the fact that it is leafless or bears only rudimentary leaves. In well-developed plants the first bald sucker is readily distinguished from the secondary axillary branches below, and is sometimes used as a convenient point from which to begin a count of the number of leaves produced by the plant.

In the following investigations a count was made of every leaf above the cotyledons. In order to do this conveniently the same leaf on each plant, counting from the cotyledons, was marked by puncturing the leaf one or two times with a heated needle. This treatment affords a quick and simple method of marking any particular leaf, and at the same time prevents the possibility of inoculating the plants with the mosaic disease.

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First Experiment

In the first experiment seed of a bagged individual plant (7*G*) of the Connecticut Broadleaf strain designated as No. 2 was used. The seed was sowed in flats Nov. 13, 1912, and on Jan. 4, 1913, the plants were transplanted to two-inch pots. On Jan. 28 they were transplanted from these pots to a bed of rich clay-loam in the greenhouse at Arlington, Virginia. From the time the plants were transferred to two-inch pots a record was kept of all the leaves produced by marking the following leaves:

4th leaf above the cotyledons marked in January.
6th “ “ “ “ “ on February 13.
8th “ “ “ “ “ “ March 1.

As soon as the young plants had become established in the bed, the moisture relations of one half of the bed were maintained at an optimum for the growth of the plants, while the other half of the bed was kept relatively dry. The moisture content of the moist soil ranged from 18 to 20 percent throughout the experiment, while that of the dry soil was kept at 10 to 12 percent.

The plants in the moist soil were as large and as vigorous in all respects as plants growing under the most favorable field conditions. The plants in the dry soil became very much stunted, reaching only half the height attained by the plants in the moist soil and blossomed about 10 days later than these.

Complete data as to the number of leaves, number of nodes, and the character of the inflorescence of the plants grown in the moist and dry soil are given in Table I.

From a comparison of the data for the two sets of plants, the following relations are brought out. From column (7), it is seen that the average number of nodes from the cotyledons up to but not including the terminal whorl of branches is the same for the normal and stunted plants, namely 32.4 and 32.9, respectively. The average number of nodes produced by the plants, inclusive of the terminal whorl, is also unchanged, namely 36.6 for the normal and 36.1 for the stunted plants. An analysis of the various elements of the inflorescence as shown in columns (4), (5) and (6) indicates somewhat different relations for the two sets of plants. From column (6) it is evident that the normal plants produced the largest number of flower branches, an average of 8.2, as compared with an average of 5.9 for the stunted plants. This reduction in the number of flower branches has taken

TABLE I

First Experiment. Number of Leaves, Number of Nodes, and Character of Inflorescence of Connecticut Broadleaf Plants Grown in the Greenhouses in Moist and Dry Soils

Moist Soil

Number of Plant	Height of Plants	No. of Leaves from Cotyledons up to, but not Including, 1st Bald Sucker	Inflorescence			Total No. of Nodes Produced not Including Terminal Whorl	Total No. of Nodes Produced Including Terminal Whorl	Date of First Blossoms in 1913	Number of Days Intervening from Date of Transplanting to Opening of First Blossom
			No. of Lateral Flower Branches, Including 1st Bald Sucker but not Including Terminal Whorl	No. of Flower Branches in Terminal Whorl	Total No. of Flower Branches, Including 1st Bald Sucker and Terminal Whorl				
1	84 in.	30	4	4	8	34	38	March 26	57
2	90 "	29	4	4	8	33	37	" "	57
3	91 "	28	5	4	9	33	37	" "	57
4	87 "	28	3	4	7	31	35	" 25	56
5	85 "	29	4	4	8	33	37	" "	56
6	90 "	28	4	4	8	32	36	" 26	57
7	96 "	28	4	5	9	32	37	" "	57
8	95 "	29	4	4	8	33	37	" 25	56
9	90 "	28	4	5	9	32	37	" "	56
10	85 "	27	4	4	8	31	35	" "	56
Av. . .	89.3 "	28.4	4	4.2	8.2	32.4	36.6		56.5

Dry Soil

Number of Plant	Height of Plants	No. of Leaves from Cotyledons up to, but not Including, 1st Bald Sucker	No. of Lateral Flower Branches, Including 1st Bald Sucker but not Including Terminal Whorl	No. of Flower Branches in Terminal Whorl	Total No. of Flower Branches, Including 1st Bald Sucker and Terminal Whorl	Total No. of Nodes Produced not Including Terminal Whorl	Total No. of Nodes Produced Including Terminal Whorl	Date of First Blossoms in 1913	Number of Days Intervening from Date of Transplanting to Opening of First Blossom
1	45 in.	31	3	4	7	34	38	March 30	61
2	42 "	30	3	4	7	33	37	" 28	59
3	48 "	30	2	3	5	32	35	April 5	67
4	48 "	30	2	3	5	32	35	" "	67
5	45 "	31	3	3	6	34	37	" "	67
6	—	30	3	3	6	33	36	" "	67
7	44 "	30	3	3	6	33	36	" "	67
8	45 "	30	3	3	6	33	36	" 1	63
9	48 "	30	2	3	5	32	35	" 4	66
10	48 "	30	3	3	6	33	36	" 6	68
Av. . .	45.9 "	30.2	2.7	3.2	5.9	32.9	36.1		65.2

place both in the terminal whorl and the lateral branches. The stunted plants produced an average of one branch less in the terminal whorl and an average of 1.3 less in the number of lateral branches (see columns 4 and 5).

As shown in column (3) the average number of leaves produced counting from the cotyledons up to, but not including, the leaf subtending the first bald sucker was 28.4 for the normal plants as compared with 30.2 for the stunted plants. Although the first bald sucker (the lowermost branch of the inflorescence) in the normal plants occupies its true position, it is evident that this branch has been suppressed in the stunted plants. Consequently, the lowermost flowering branch appearing upon the stunted plants no longer represents the true position of the first bald sucker. More properly, it is one of the higher lateral branches of the inflorescence which now appears to be the first bald sucker.

In a second experiment the true as compared with the apparent expression of the first bald sucker is even more clearly and strikingly shown.

Second Experiment

In the first test stunting was brought about by maintaining a very dry soil. In the second test, the plants were grown in pots, and stunting was brought about by growing one set of plants in very small pots containing relatively little soil.

The plants used for this test were obtained from a single mother plant, and are direct descendants through three stunted generations of one of the stunted individuals of the preceding test.

The seed was sown in flats Jan. 7, 1915. As soon as the plants were large enough, they were transplanted from the flats directly to 8-inch and 4-inch pots respectively, in which they remained throughout the experiment. In this test 17 plants were grown in the 8-inch and 23 plants in the 4-inch pots. Owing to the fact that the plants in the smaller pots were very severely stunted, they grew very slowly and blossomed about two months later than the plants grown in the larger pots. Complete data as to the number of leaves, number of nodes, character of the inflorescence, etc., for the two sets of plants are given in Table II.

From the data given in Table II it is evident that the plants were stunted much more severely than in the preceding test. From column (8) it is shown that the average number of nodes produced, not including the terminal whorl, is the same for both sets of plants, namely 32.4 for the plants in the large pots, and 32.8 for the smaller plants. From column (9) it is also shown that the average number of nodes produced, including the branches of the terminal whorl, is the same, *i. e.*, 35.8 for the larger plants and 35.6 for the smaller plants. From column (7) it is evident that the average number of flower branches

TABLE II

Second Experiment. Number of Leaves, Number of Nodes and Character of Inflorescence of Connecticut Broadleaf Plants Grown in Pots in the Greenhouse at Arlington, Virginia, 1915

Plants in 8-inch Pots

No. of Plant	Height to Top of Flower Head	Number of Dead Basal Leaves Above Cotyledons	Number of Leaves from Cotyledons to, but not Including First Bald Sucker	Inflorescence			Total Number of Nodes Produced, not Including Terminal Whorl	Total Number of Nodes Produced Including Terminal Whorl	Date of First Blossoms in 1915	Number of Days Intervening from Date of Transplanting to Opening of First Blossom
				No. of Lateral Flower Branches Including 1st Bald Sucker, not Including Terminal Whorl	Number of Flower Branches in Terminal Whorl	Total Number of Flower Branches Including First Bald Sucker and Terminal Whorl				
1	39 in.	1 to 10 inc.	27	5	4	9	32	36	Apr. 21	57
2	37 "	do.	30	5	3	8	35	38	" 28	64
3	43 "	do.	25	6	3	9	31	34	" 21	57
4	47 "	1 to 9 inc.	27	5	4	9	32	36	" 21	57
5	48 "	1 to 10 "	25	7	3	10	32	35	" 21	57
6	44 "	1 " 12 "	26	7	4	11	33	37	" 21	57
7	45 "	1 " 10 "	24	7	3	10	31	34	" 21	57
8	42 "	1 " 8 "	26	7	3	10	33	36	" 21	57
9	45 "	1 " 10 "	27	6	3	9	33	36	" 21	57
10	46 "	1 " 10 "	26	6	4	10	32	36	" 22	58
11	48 "	1 " 11 "	25	6	4	10	31	35	" 23	59
12	45 "	1 " 10 "	26	6	4	10	32	36	" 22	58
13	38 "	1 " 8 "	26	7	4	11	33	37	" 26	62
14	48 "	1 " 9 "	26	7	3	10	33	36	" 28	64
15	43 "	1 " 10 "	27	7	3	10	34	37	May 1	67
16	36 "	—	25	5	3	8	30	33	" 2	68
17	48 "	—	31	3	3	6	34	37	Apr. 30	66
Av. . .	43.6 "		26.4	6	3.3	9.4	32.4	35.8		60.1

Plants in 4-inch Pots

No. of Plant	Height to Top of Flower Head	Number of Dead Basal Leaves Above Cotyledons	Number of Leaves from Cotyledons to, but not Including First Bald Sucker	No. of Lateral Flower Branches Including 1st Bald Sucker, not Including Terminal Whorl	Number of Flower Branches in Terminal Whorl	Total Number of Flower Branches Including First Bald Sucker and Terminal Whorl	Total Number of Nodes Produced, not Including Terminal Whorl	Total Number of Nodes Produced Including Terminal Whorl	Date of First Blossoms in 1915	Number of Days Intervening from Date of Transplanting to Opening of First Blossom
1	18 in.	1 to 13 inc.	No lateral branches	0	4	4	31	35	June 24	122
2	20 "	1 " 14 "	"	0	3	3	31	34	" 21	119
3	20 "	1 " 14 "	"	0	3	3	31	34	" 20	118
4	16 "	1 " 13 "	"	0	3	3	31	34	" 24	122
5	22 "	1 " 14 "	"	2	3	5	34	37	" 18	116
6	25 "	1 " 14 "	32	1	3	4	33	36	" 16	114
7	20 "	1 " 15 "	No lateral		3	3	33	36	" 20	118
8	25 "	1 " 14 "	32	1	3	4	33	36	" 18	116
9	26 "	1 " 14 "	30	1	3	4	31	34	" 21	119
10	29 "	1 " 13 "	29	1	3	4	30	33	" 24	122
11	17 "	1 " 17 "	31	1	2	3	32	34	—	——
12	25 "	1 " 17 "	34	1	3	4	35	38	—	——
13	17 "	1 " 17 "	No lateral	0	3	3	32	35	—	——
14	19 "	1 " 17 "	"	0	2	2	33	35	—	——
15	21 "	1 " 17 "	34	1	3	4	35	38	—	——
16	17 "	1 " 17 "	No lateral	0	2	2	34	36	—	——
17	18 "	1 " 17 "	"	0	3	3	35	38	—	——
18	19 "	1 " 17 "	"	0	2	2	34	36	—	——
19	20 "	—	33	1	2	3	34	36	—	——
20	18 "	—	33	1	3	4	34	37	—	——
21	18 "	—	No lateral	0	2	2	33	34	—	——
22	19 "	—	"	0	3	3	34	37	—	——
23	22 "	—	32	1	4	5	33	37	—	——
Av. . .	20.4"		32	1.0+	2.8	3.3	32.8	35.6		118.6

in the entire inflorescence is considerably higher for the plants in the large pots, *i. e.*, 9.4 as compared with only 3.3 for the smaller plants. The average number of branches in the terminal whorl is somewhat higher for the larger plants, 3.3 for the plants in the 8-inch pots, and 2.8 for the plants in the 4-inch pots. With respect to the position of the first bald sucker, columns (4) and (5) bring out the same relations that were shown in the preceding test. Although an average of 6 lateral flower branches was produced by the larger plants, the average had been reduced to one lateral branch in the plants grown in 4-inch pots.

Owing to the fact that stunting has been extreme in this test, the data in column (4) are particularly significant. For the larger plants the average number of leaves above the cotyledons, not including the first bald sucker, is 26.4 as compared with the much higher average of 32 for the ten plants in the 4-inch pots. In many of these plants it is shown that there was complete suppression of all the lateral branches of the inflorescence.

A comparison of the data for the two experiments, as shown in Tables I and II, brings out the fact that the average number of nodes produced by the plants, exclusive of the terminal whorl of branches, has remained unchanged under all conditions of growth:

1st experiment	Plants moist soil,	32.4 nodes
	" dry "	32.9 "
2d experiment	" 8-inch pots,	32.4 "
	" 4 " "	32.8 "

If the average number of nodes, including the terminal whorl, is considered, it is indicated that the plants in the second experiment have produced somewhat fewer nodes than those in the first experiment:

1st experiment	Plants in moist soil	36.6 nodes
	" " dry "	36.1 "
2d experiment	" " 8-inch pots	35.8 "
	" " 4 " "	35.6 "

Since it has been shown that the average number of nodes exclusive of the terminal whorl has been unchanged, it is evident that the above reduction has taken place in the branches of the terminal whorl.

Unfavorable conditions reduce the size of the inflorescence quite as readily as the size of the leaves. In severely stunted plants the branches of the terminal whorl become so greatly reduced that they may be represented by single blossoms as was the case with many plants grown in the 4-inch pots in the second experiment. (See Plate XX.)

In plants stunted even more severely than those in the 4-inch pots in the second experiment, the terminal whorl is completely suppressed, and the inflorescence of the plant is reduced to the single terminal blossom of the main stem. Such plants were only 8 to 10 inches in height when blossoming finally took place, and the majority of the leaves were reduced to mere bracts.

Experiments have shown that tobacco plants may be very considerably stunted before any retardation in time of blossoming takes place. Beyond these limits, however, blossoming in severely stunted plants may be delayed almost indefinitely. In fact, in the writer's experience, it has been possible to keep young tobacco plants in a practically dormant condition for periods as long as the normal life of the plant. Plants kept in a practically dormant condition for about 5 months are shown in Plates XXI–XXIII. The smallest plants in the 2-inch pots are sister plants of the same age as the large plants, and all were grown from seed sowed Nov. 13, 1912. Experiments with these dormant plants have shown that this inhibition of growth is only temporary in its effects. If such plants are transplanted to the field, vigorous growth ensues and plants of nearly normal size are produced.

In the field it is known that the number of commercial leaves is more or less variable from year to year. These variations depend upon such accidental factors as depth of transplanting, drought, height of topping, etc., and do not show the true number of leaves produced by the plants. In ordinary field practice probably not less than 6 or 8 leaves above the cotyledons are lost. As shown in Table II, the plants grown in the 8-inch pots lost from 8 to 10 leaves above the cotyledons, while those more severely stunted in the 4-inch pots lost in some instances as many as 17 leaves.

From the writer's experimental data in Tables I and II it is indicated that the number of nodes below the terminal bud is not changed by environmental conditions.

Hayes, East and Beinhart[2] from a statistical study of some New England types in the field, came to the conclusion that environmental conditions have little effect upon the number of leaves produced in the field.

Although in the writer's experiments it is indicated that severe stunting brings about a suppression of the flower branches of the terminal whorl, it is probable that the terminal bud does not appear until the number of nodes preceding it and predetermined in the embryo have developed.

From field experiments with tobacco East and Hayes (Am. Naturalist, **48**: 5–48, previously cited) have concluded that the conditions under which the mother plants grow may determine the number of leaves in the embryo within the normal limits of fluctuation. To establish this point beyond question, however, it will be necessary to determine all the nodes produced by the plants beginning from the cotyledons. It is clear that results based upon counts from the first bald sucker may not be entirely free from error. From the data for the experiments given in Tables I and II, it appears that the position of the first bald sucker has not been constant under all conditions.

Thus, counting from the cotyledons up to, but not including, the first bald sucker, the normal plants in experiment I produced an average of 28.4 leaves. In the second experiment the average for a similar count was only 26.4, amounting to a difference of two leaves. Counting all the nodes below the terminal blossoms, however, the average for both sets of plants was the same, namely 32.4 for the plants in experiment I and 32.4 for those which averaged only half as tall in experiment II. As the limits of minimum growth are approached it is evident that the first bald sucker can no longer be relied upon for comparative studies.

Between the limits of minimum growth and maximum growth all degrees of ontogenetic expression with respect to capsule and seed development may be noted. Reduction in size of plant is accompanied by a more or less proportionate decrease in the number of seed pods borne by the plant.

It has not yet been shown that the physiological environment

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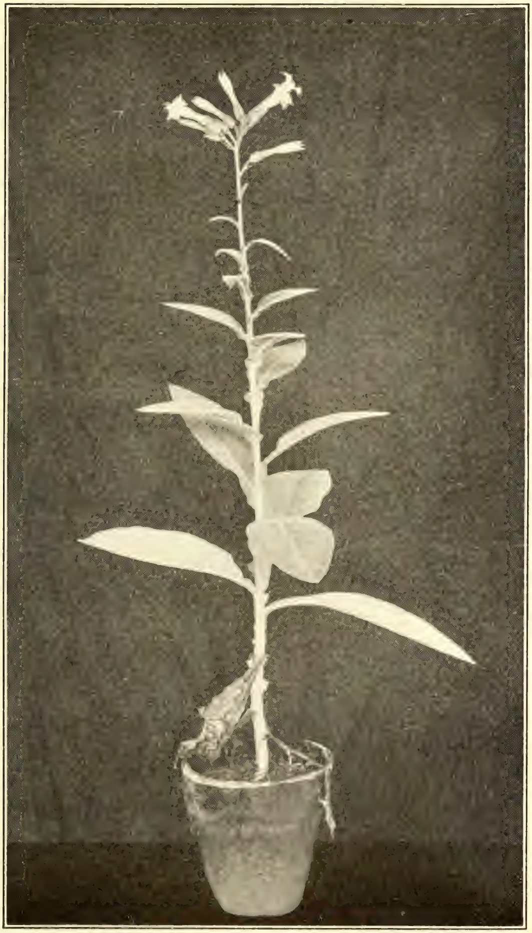
[2] Hayes, H. K., East, E. M., and Beinhart, E. G., "Tobacco Breeding in Connecticut." Conn. Agr. Exp. Sta. Bull. 176: 40–42. May, 1913. See also, East, E. M., and Hayes, H. K. "A Genetic Analysis of the Changes Produced by Selection in Experiments with Tobacco." American Naturalist, **48**: 5–48. 1914.



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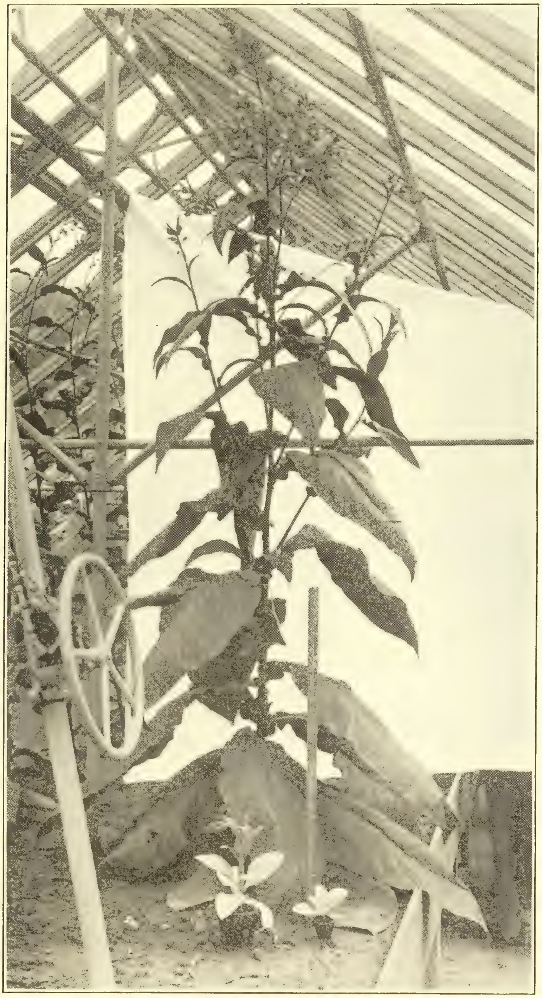

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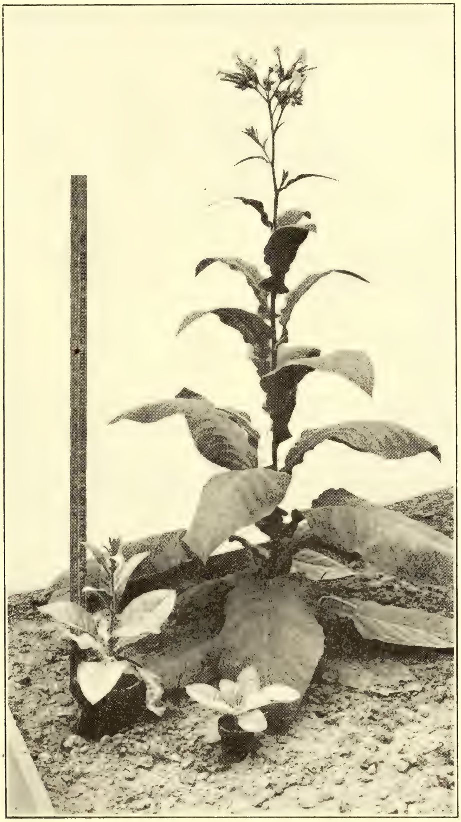
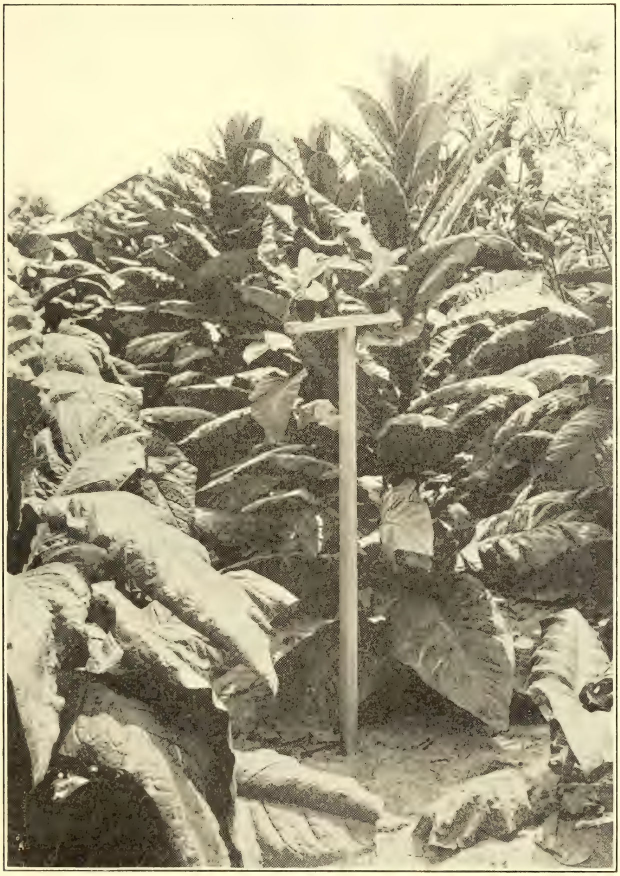


ALLARD: EFFECT OF ENVIRONMENT ON TOBACCO PLANTS.



ALLARD: EFFECT OF ENVIRONMENT ON TOBACCO PLANTS.

furnished by a stunted plant capable of supporting but one capsule is any less favorable to normal seed development than that furnished by a plant of maximum size producing great numbers of capsules. If it is assumed that nutritional differences during some initial period of embryonic development determine leaf number, it is reasonable to expect that capsules developed at different stages of the reproductive period on the most completely nourished plants will show a slightly higher or lower leaf number.

Summary

Very severely stunted tobacco plants have been studied in comparison with plants of normal size grown under optimum conditions of soil moisture. The average number of nodes produced above the cotyledons, exclusive of the branches of the terminal whorl, has remained constant under all conditions. Unfavorable conditions reduce the size of the inflorescence. Stunting may be carried to such an extreme that the branches of the terminal whorl may be entirely suppressed, so that the inflorescence of the plant is reduced to the single terminal blossom of the mainstem.

The position of the first bald sucker is not constant under all conditions. Although it may occupy its true position in normal plants, it is one of the higher lateral branches of the inflorescence which appears to be the first bald sucker in stunted plants. In very severely stunted plants, all the lateral branches of the inflorescence may be suppressed.

Office of Tobacco and Plant Nutrition Investigations,
Bureau of Plant Industry,
U. S. Department of Agriculture

EXPLANATION OF PLATES XX–XXIII

Plate XX. Stunted Connecticut Broadleaf Plant grown in 5-inch pot.
Plate XXI. Plant No. 7 grown in moist soil in Experiment I.
Plate XXII. Plant No. 7 grown in dry soil in Experiment I.
Plate XXIII. Plant of 1st generation of cross Md. Mammoth ♀ × 70-Leaf Cuban ♂.

OENOTHERA MUTANTS WITH DIMINUTIVE CHROMOSOMES[1]

Anne M. Lutz

A. Introduction

For a number of years the writer has devoted considerable attention to the study of the somatic chromosomes of *Oenothera Lamarckiana* and its derivatives in an effort to ascertain whether or not each species or type is represented by a definite, fixed number of chromosomes. Having found and announced (Lutz, '12) that all forms having identical somatic characters throughout all stages of their development invariably have the same number of chromosomes, we may now consider some of the results of these studies in detail. This report may be considered the first of a series of three dealing with somatic chromosome number in mutants of the Lamarckiana group of Oenothera.[2]

During the first four years investigations were conducted at the Station for Experimental Evolution,[3] Cold Spring Harbor, where careful work on an extensive scale was made possible by the kindness and generosity of the director, Professor Charles B. Davenport.

Owing to the nature of the study, a thorough knowledge of the somatic characters of each individual became, clearly, a matter of first importance; therefore, from the time of the appearance of the seedling leaves until the end of the fruiting period late in October, attention was directed almost exclusively to the careful study of the somatic characters of the growing plants.

Tips of rapidly growing roots from rosettes in 3-inch pots, supplemented by various somatic tissues of young buds, were employed for the determination of somatic chromosome number.[4] All, with the exception of a few of the first, were fixed with chrom-acetic. One fixing fluid was employed throughout in order that reliable conclusions might be reached concerning the comparative sizes and shapes of chromosomes of various types. Fixations were made at all hours, but only those prepared between 10 A.M. and 1 P.M. offered satisfactory material for study. Tips were embedded after 5 minutes in melted paraffin and cut transversely for chromosome counts, usually about 7 μ thick. Great care was taken to preserve an unbroken series. When this was impossible, the point of interruption was carefully indicated by a diamond mark on the slide. This precaution was strictly observed, even when a single section was mounted upside down. All material was stained with Heidenhain's iron-haematoxylin.

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[1] Reported in a paper read before the Botanical Society of America, December 29, 1915.

[2] To be published in this Journal.

[3] Maintained by the Carnegie Institution of Washington until March, 1911.

[4] For the sake of brevity, the rather awkward term of "chromosome number" will be substituted frequently for "number of chromosomes" throughout this report and others to follow.

The chromosome numbers of mutant types to be announced by the writer in the papers of this series, as well as those of forms mentioned in previous reports (with very few exceptions, notable among which is *O. gigas*), were determined from fixations prepared from the mutant, and not from offspring of the mutant duplicating the characters of the parent, although counts were also made from these when the opportunity was presented.

In order to be assured of the correct identification of the Cold Spring Harbor mutants supposed to duplicate the characters of forms which de Vries had described and named, I visited Professor de Vries's gardens at Amsterdam in July and September, 1911, and in June, 1912, where, through the courtesy of Professor de Vries, I had the privilege of studying forms of particular interest as greenhouse rosettes, and, later, as flowering plants. The distinguishing characteristics of important forms were carefully noted and later compared with descriptions and photographs of Cold Spring Harbor types supposed to duplicate them.

From time to time, Professor de Vries has greatly assisted in the elucidation of obscure points by furnishing the writer with careful and detailed descriptions of certain forms whose identity, on American soil, had not been clearly established; by giving additional information, when perplexing questions arose, regarding certain forms and cultures mentioned in his publications; and by providing the writer, on many occasions, with generous supplies of seeds of important and significant forms. It is with the keenest sense of my obligations to Professor de Vries that I express my gratitude for his never-failing kindness.

It was my highly esteemed privilege to compile the results of the

Cold Spring Harbor studies and to work out the majority of the details of special cell-study connected with these investigations (exclusive of chromosome counts) in consultation with Professor V. Grégoire at the University of Louvain during the year 1911–12. It is with the most profound gratitude that I express my appreciation of the help received from Professor Grégoire in this work—a portion of which is represented by these three communications; of his untiring devotion of time and thought from October, 1911, until August, 1914, to the correct interpretation of recorded observations; to the verification of numerous microscopical details of the work; and to the solution of many problematical questions which have arisen in connection with these studies. The chromosome count of every type mentioned by the writer in this and the two reports to follow, was carefully verified by Professor Grégoire, especial attention having been given to those which for any reason might be questioned. It is reasonably certain, therefore, that the identification of the Cold Spring Harbor forms and the chromosome numbers to be announced in the three papers of the series, are correct.

To Professor J. C. Arthur, Professor Stanley M. Coulter and Mr. George N. Hoffer, I am most deeply indebted for innumerable courtesies and many privileges enjoyed in the botanical laboratories of Purdue University during the past three years.

I wish also to express my gratitude to Professor H. E. Crampton for privileges accorded to me, through his courtesy, at the American Museum of Natural History; to Dr. F. E. Lutz and to Professor B. M. Davis for numerous favors and accommodations; to Professor H. H. Bartlett for generously providing me with unpublished data relating to certain new and interesting forms which he has recently discovered, and for his kindness in giving me permission to quote his statements concerning them in these communications.

The primary object of these three papers is to discuss, in the light of the Cold Spring Harbor and Louvain studies of somatic chromosome number in *Oenothera Lamarckiana* and its derivatives, certain theories and conclusions which Gates has announced from time to time, and which Gates and Miss Thomas have based upon the results of their investigations. Partly for this reason, but chiefly because of limitations of space, all but a few of the more important mutants reported will be described very briefly, or not at all, these descriptions being reserved for a later publication. With the exception of a few forms which are of particular interest, those which have appeared but once or twice or which have appeared more frequently, but in every case have failed to come to flower, will be designated temporarily by type numbers only.

It may be understood that all facts and theories embodied in the reports of this series pertain only to the plants of the *Lamarckiana* group, unless otherwise stated.

As a preliminary to the study of 14+-chromosome forms, we may briefly consider the 14-chromosome mutants which have appeared in the Cold Spring Harbor cultures.

B. 14-CHROMOSOME MUTANTS

Five hitherto undescribed types, whose chromosome numbers have not been previously announced, appeared in cultures of *O. Lamarckiana*, *O. lata*, *O. nanella*, etc. All were found to have 14 chromosomes.

(1) Type 2787 and (2) type 2803 are unimportant forms and may be passed by for the present.

(3) Type 3539, found in a 1908 culture of *O. Lamarckiana*, had narrow, asymmetrical leaves, a character which persisted throughout life. The majority of the flowers had 4+-rayed stigmas. The somatic chromosome number of this plant was ascertained in 1909.

(4) *O. plicatula* appeared among the offspring of a selfed *lata* mutant in 1909. It was very beautiful and thrifty in appearance. Coming to flower as an annual, it attained a height of about 9 dm. when full grown. As in the case of *O. Lamarckiana* and other forms, a circlet of rosette branches was given off (text fig. 1), those of the mutant ascending more rapidly than the rosette branches of *O. Lamarckiana*. The buds were quadrangular, the sepals attaining the deep red of *rubrinervis*. The open flowers measured about 7–8 cm. in diameter, as a rule, and readily attracted the eye by the peculiar markings shown in the accompanying photographs (text figs. 1 and 2; compare the latter with a flower from *O. Lamarckiana*, text fig. 3). These troughs or ridges, as they may be called, according to the surface of the petal from which they are viewed, result from a somewhat too snug fit of the quadrangular cone of petals within the quadrangular cone of sepals. Since the distance between the angles of neighboring sepals appears to be less, in such instances, than the distance between the angles of the petal

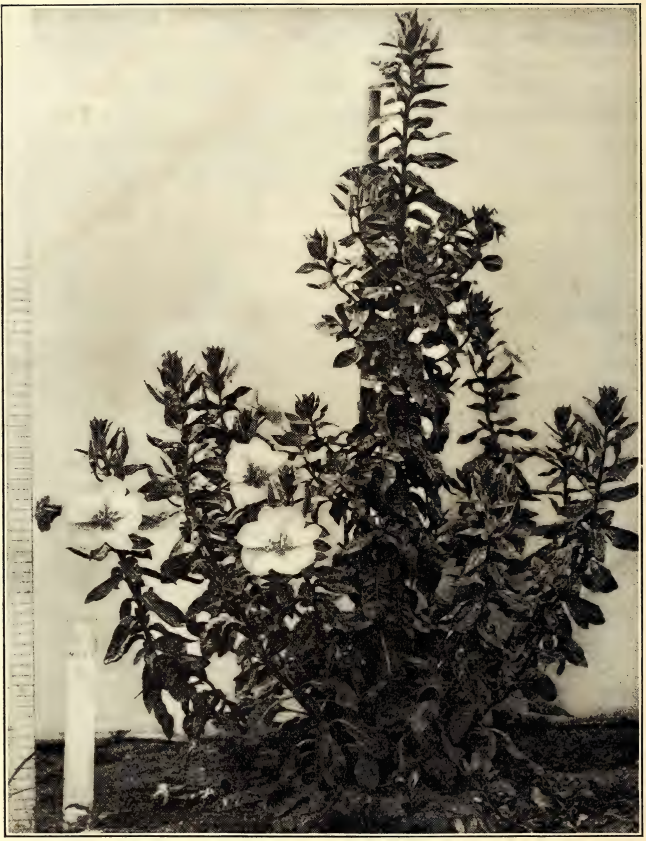

FIG. 1. *O. plicatula*, plant No. 4555, C. S. H., 1909. Mutant offspring of *O. lata*, selfed. Photographed August 25, 6:40 A. M. Flowers with "crimped" petals.

cone directly beneath, the petals are pushed into a sort of trough (whose convexity must appear upon the stigma surface of the petals) extending in the same direction as the line marking the union of two neighboring sepals.

Markings of this sort, though less pronounced and often more irregular in appearance, are found in the newly-opened flowers of many forms, but they usually disappear shortly after the petals are released. The crimps or ridges in the petals of *O. plicatula* persisted as shown in text figure 2 until the flowers faded. The flowers shown in text figures 2 and 3 were photographed in the early morning of the same day.

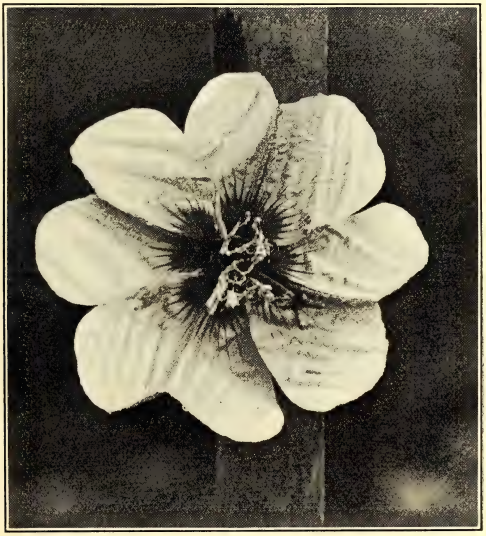

FIG. 2. *O. plicatula*; flower taken from plant shown in Text-fig. 1. Photographed September 19, 6:40 A. M., 1909. Showing "crimped" petals.

While *O. plicatula* could not be mistaken for *O. rubrinervis* under any circumstances, a type grown at Cold Spring Harbor which may have been identical with de Vries's *rubrinervis*, had crimped petals and red sepals (text fig. 4).[5] In 1913 a few plants were grown from seeds of the Amsterdam *rubrinervis*, kindly sent to me by Professor de Vries, and the single individual which came to flower had crimped petals.

O. plicatula produced an abundance of seemingly good pollen consisting of 3-lobed grains. Unfortunately, no effort was made to self this mutant.

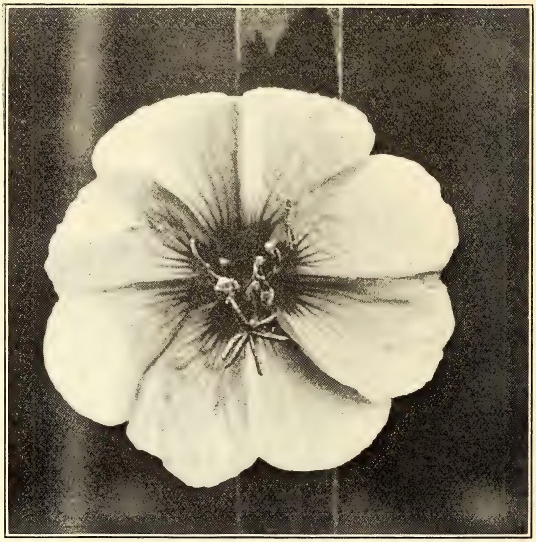

FIG. 3. *O. Lamarckiana*, plant No. 4650, C. S. H., 1909. Offspring of *O. lata*, selfed. Photographed September 19, 6:50 A. M., same day and hour as flower shown in Text-fig. 2. Showing absence of "crimps" in petals.

(5) *O. delicatula*, a form of especial interest, will be fully described in a later communication.

(6) Type 3514 (described on page 520 of this report) may be a modified form of de Vries's *rubrinervis*. While the chromosome number of this form has not been announced previous to this communication (un-

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[5] This character is much more pronounced in some individuals than in others.

less the *rubrinervis* in which Gates found 14 chromosomes duplicated this type instead of de Vries's mutant), the type is not a new one, having been figured by MacDougal as early as 1905.

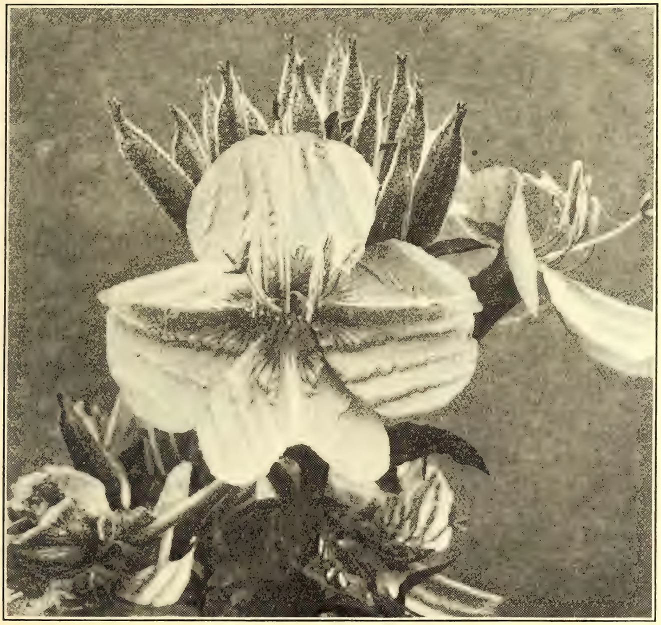

FIG. 4. *O. rubrinervis* (?), plant No. 3903, C. S. H., 1908. Flower photographed in early morning, showing "crimped" petals.

C. 14^{+1}-CHROMOSOME MUTANTS

1. *Recent discoveries indicative of the production of 7^{+1}-chromosome gametes by 14- and 15-chromosome forms*

The discovery of chromosome degeneration in 14+-chromosome forms by Geerts ('11), together with later studies of this peculiar irregularity in 15-chromosome forms by Gates and Miss Thomas ('14), has thrown a flood of light upon the origin of the small chromosome in 14^{+1}-chromosome offspring of 15-chromosome *O. lata* × 14-chromosome *O. Lamarckiana.*

Chromosome degeneration was observed by Geerts in 21-chromosome offspring of *O. lata* × *O. gigas*, *O. Lamarckiana* × *O. gigas* and *O. gigas* × *O. Lamarckiana*, all from de Vries's gardens. On the heterotypic spindles of the individuals which he studied he found 7 pairs of whole chromosomes and 7 which were unpaired. With the first division the two members of each of the 7 pairs separated and passed to opposite poles, while 3 of the 7 unpaired members of the group usually moved towards one pole and 4 towards the other. As the 7 chromosomes approached the pole the longitudinal split, preparatory for the second division, became quite distinct, but was less clear and more irregular in the 3 or 4 remaining chromosomes. Sometimes, also, the latter failed to reach the pole and were not included within the daughter nucleus. Later, 7 clearly split chromosomes and 3 or 4 small ones showing the longitudinal split less clearly, were found at the equator of each of the two homotypic spindles. Thus, to each of the four poles were distributed 7 distinct chromosomes and often a number of irrgeular chromosomes or pieces of chromosomes. In regard to the fate of these fragments or irregular bodies, he says (italics not employed in the original): "*Wenn die Tetradenkerne entstehen, wird bisweilen dieses Chromatin in den Kern aufgenommen,* aber meistens liegen ausserhalb der Kernwand auch Chromatinteilchen. Diese Chromatinstücke, welche ausserhalb der Kerne zurückgelassen werden, entwickeln sich oft zu Zwergkernen, in den jungen Pollenkörnern sowie in dem jungen Embryosacke. Beim Auswachsen des Embryosackes verbleichen diese Zwergkerne und verschwinden allmählich."

From Gates and Miss Thomas (pp. 538–539) we learn that ". . . in the heterotypic mitosis in the *lata* and *semilata* forms, the 15 chromosomes are usually distributed so that 7 go to one daughter-nucleus and 8 to the other. In rare cases the distribution is 9 and 6. . . . More frequently the extra chromosome is left behind, where it may be seen fragmenting and degenerating in the cytoplasm."

A remarkable irregularity in 15-chromosome *O. lata rubricalyx* is described by Gates and Miss Thomas (pp. 541–542) and the statements which follow are of particular interest in view of the fact that the Cold Spring Harbor and Louvain studies have demonstrated the actual existence of 14^{+1}-chromosome mutants:

"The left-hand group contains 7½ chromosomes, while the right-hand group consists of 6 whole chromosomes, a half-chromosome and a small fragment. There is in addition a chromosome in the cytoplasm near this group, which has been left out of the nucleus in interkinesis. This makes the full quota of 15 chromosomes. The origin of the fragment is obscure, but the figure shows that even fragments of chromosomes may be distributed occasionally to the daughter-nuclei. Unless afterwards extruded from the pollen nuclei, such fragments will no doubt affect the later development of the individual; they might remain independent or become attached to or fused with one of the other chromosomes."

Thus the researches of Geerts have shown that during the female, as well as male, reduction in certain 21-chromosome forms, chromosomes in excess of 14 may fragment and degenerate, while the researches of Gates and Miss Thomas have demonstrated that one of the chromosomes of certain 15-chromosome forms may fragment and degenerate during the reduction divisions of the pollen mother cell. These facts, together with other evidence which will be offered by the writer later, suggest the conclusion that *chromosome degeneration occurs in all forms having 14+-chromosomes, though probably less frequently in plants having 28, than in other 14+-chromosome forms particularly if the 28 represent a double set of the original 14. In other words, there appears to be a tendency in 14+-chromosome forms, to return to the original 14-chromosome condition; furthermore, this tendency appears to be more pronounced in forms having 21 chromosomes, than in those having a double set of the original 14.* It may be that sometimes a portion, sometimes all, of the chromosomes in excess of 14 degenerate, and that gametes of 14+-chromosome plants are equipped with various irregular numbers of chromosomes.[6] In addition to the whole 7 or 7+-chromosomes present in the germ-cells of such forms, we may expect to find, occasionally, one or more fragments of chromosomes.

2. *14^{+1}-chromosome offspring of O. lata × O. Lamarckiana*

A 14^{+1}-chromosome condition has been observed by the writer in 3 plants. The first, No. 3878, was found among the F_1 offspring of *O. lata* × *O. Lamarckiana* grown at Cold Spring Harbor in 1908 and the second, No. 4605, in another culture of the same cross in 1909.

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[6] Doubtless more frequently true of 21-, than of 15- and 28-chromosome forms.

The second mutant duplicated the vegetative characters as well as the somatic chromosome number of the first; therefore the type represented by these two individuals will be known as *O. aberrans.*

O. aberrans (text fig. 5) attained the height of *O. Lamarckiana,* thereby exceeding the height of the female parent. It produced a number of long rosette branches which were somewhat more decumbent than those of *Lamarckiana.* The buds, which remained yellow throughout the entire season, were tapering and slender. A large number of flowers were produced daily, 86 having been counted on No. 4605 one

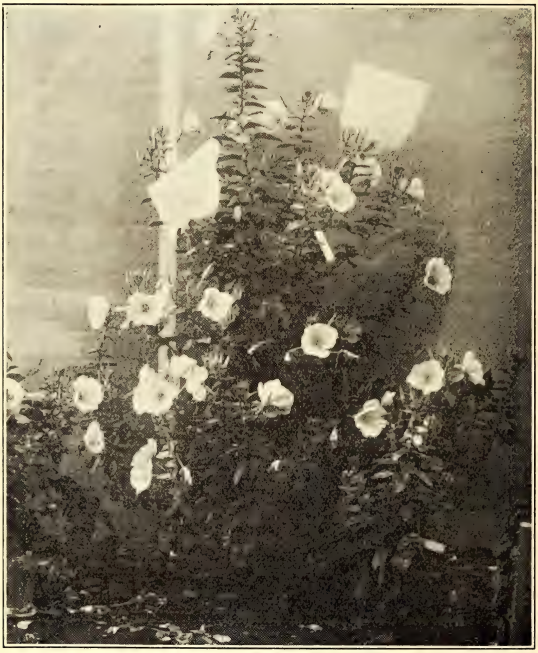

FIG. 5. *O. aberrans,* plant No. 3878, C. S. H., 1908. 14^{+1}-chromosome mutant offspring of *O. lata* × *O. Lamarckiana.*

day near the height of the flowering season. The petals were smooth and a slightly lighter yellow than is characteristic of *O. Lamarckiana.* A portion of the buds produced a moderate abundance of pollen containing relatively few seemingly good grains and still others none at all. It was almost impossible to obtain seed from the self-pollination of this form.

The somatic chromosomes of the 1908 mutant (No. 3878) were studied and the number determined in the autumn of 1909. Finding that this plant had 14 chromosomes of the usual size and 1 small one, it was recalled that a second mutant (No. 4605) of the same type had been produced by *O. lata* × *O. Lamarckiana* in the season which had just passed; therefore root-tips from this plant were sectioned and studied with much interest, and here also 14 chromosomes of the usual size and 1 small one were found (late autumn of 1909).

Unfortunately, but one fixation of 16 tips had been prepared from No. 3878 and one of 20 tips from No. 4605. These were not wholly satisfactory for study, as the fixations were poor and only comparatively few figures were found in perfect metaphase (the only satisfactory stage for the determination of chromosome number in root tips of *Oenothera*). However, the tendency of the small chromosome to occupy a peripheral position on the equatorial plate and to be alloted the full space of a large chromosome, often made it possible to determine the presence of the small member when it was impossible to count the long ones.

In transverse sections of root-tips from No. 4605, 25 metaphase figures were observed, showing the small chromosome with unmistakable clearness. In but 8 of this number were the large chromosomes sufficiently well separated to make it possible to count them accurately, but in each of the 8, 14^{+1} were clearly demonstrated (figs. 2 and 3).

In No. 3878, 59 figures were observed in which the small chromosome was clearly identified. In 30 of the 59 the chromosomes were sufficiently well separated to enable one to determine the number precisely, and in each of the 30, 14^{+1} were counted (fig. 1).

Although in both plants the small chromosome occupied a peripheral position on the equatorial plate in the majority of cases (figs. 1 and 3), its position was by no means constant, as it was found farther in occasionally (fig. 2).

In 1911 and 1912 I had the privilege of preparing fixations for the determination of somatic chromosome number from many interesting forms in Professor de Vries's garden at Amsterdam. One of these, No. 6082, was a mutant in a *lata* × *Lamarckiana* culture identified by Professor de Vries as a typical *O. rubrinervis*. Lutz ('07)[7] and Gates ('08) had reported 14 chromosomes for *rubrinervis;* it was a matter of considerable surprise, therefore, to find 14 chromosomes of the ordinary size and 1 small one in the root-tips of this plant. Notwithstanding the fact that but one fixation of 13 tips had been prepared from de Vries's *rubrinervis*, these chanced to be exceedingly well preserved and to show an abundance of good figures in metaphase; therefore the somatic chromosomes of this plant were studied with the greatest care by Professor Grégoire and myself in 1912.

A total of 125 metaphase figures showed the small body distinctly, although the longer ones were too massed and tangled to count with accuracy. In 52 figures 14 chromosomes of the usual size and 1 small one were distinctly counted. As in the preceding forms, the small body usually occupied a peripheral position on the equatorial plate and was commonly alloted the full space of a large chromosome (figs. 4 and 5. See also figs. 1, 2, 3). It was sometimes found farther in, however, and its position in the group was by no means constant. Usually, the small body stood out with remarkable clearness, but not infrequently the chromosomes were too massed and tangled to determine whether or not the small body was present. Sometimes it appeared to be absent, but careful focusing revealed it lying very close beside one of the longer chromosomes or in the apex of a V, formed by contact of the ends of two chromosomes. In several cells of each of the three plants the long chromosomes appeared to be sufficiently well separated to reveal the small body, had it been present, yet it was not found. In such cases it may have been absent, hidden by one of the longer chromosomes, or torn out of position by the knife in sectioning. Such figures were comparatively rare, however, and the large number of cases in which the small body was recognized in groups of tangled chromosomes where one would expect it to be hidden, was one of the striking features of these plants.

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[7] In a paper read at the Seventh International Zoological Congress in 1907 (published in the Proceedings of the Congress in 1910) the writer announced 14 chromosomes for *O. rubrinervis* offspring of open-pollinated *O. rubrinervis*. It is now well known that, in addition to the mutants *O. lata*, *O. oblonga*, *O. rubrinervis*, etc., *O. Lamarckiana* and certain other forms produce *lata*-like, *oblonga*-like, *rubrinervis*-like, etc., mutants which may be readily mistaken for the true types by inexperienced workers. Later cultures have shown that several of my first season's identifications (1907) were erroneous ones of this sort (see second paper of this series). Since the "*rubrinervis*" offspring above referred to were grown in 1907, the possibility of error in identification is conceded. Seeds of *O. rubrinervis* de Vries were received from Professor de Vries in 1913, but having in my possession no seeds of the strain from which the 1907 parent was derived, I have been unable to compare the two forms directly. The original "*rubrinervis*" mutant was produced at the New York Botanical Garden. Re-examination of the sections of root-tips from which the original counts were made revealed many beautiful metaphase figures with 14 chromosomes, but none with 14^{+1}.

3. *Origin of the Small Chromosome*

Gates and Miss Thomas occasionally found chromosomes fragmenting and degenerating on the heterotypic and homotypic spindles of 15-chromosome forms. Sometimes, also, a chromosome of the heterotypic group in a 15-chromosome form was seen to be pulling irregularly apart in the middle, leaving strands of chromatin matter trailing between the separating portions. The same peculiarity was observed in a 14-chromosome *Lamarckiana*-like offspring of a 15-chromosome plant. The authors believe that this indicated a pathological condition of the chromosomes concerned and that probably they would have degenerated later.

The two 14^{+1}-chromosome plants having identical vegetative characters (*O. aberrans*, Nos. 3878 and 4605) had the same type of small chromosome, namely, a body which was quite short and even more slender, it sometimes appeared, than the long members of the group. On the other hand, the 14^{+1}-chromosome *O. rubrinervis* had a different type of small chromosome—a body which was no longer than the small chromosome of the two preceding plants, but which seemed to be even broader than the other members of the group, at least in some instances. These conditions are particularly interesting, in view of the fact that these two mutant types were strongly contrasted in so many of their vegetative characters and were about as unlike as it is possible for two forms to be.

The small body of these two types of 14^{+1}-chromosome mutants may have arisen in the same or in a different manner. One or both may have represented merely a fragment of a degenerating chromosome, such as Gates and Miss Thomas observed on heterotypic and homotypic spindles, or a remnant of one of the two halves of a heterotypic chromosome which had pulled apart in the middle. It is even conceivable that the slender body of *O. aberrans* may have been merely a remnant of a trail of chromatin matter or a remnant of one of the very interesting "half-chromosomes" which they describe—the half of a chromosome which has separated from its mate on the heterotypic, instead of the homotypic, spindle, and which the authors believe degenerate later, in many cases. The small body in de Vries's *rubrinervis* may have been merely a piece, which by some process had become detached from one of the long chromosomes. This raises the question, Have we any evidence to indicate that pieces sometimes become detached from chromosomes?

Gates and Miss Thomas state (p. 537) that several cases were found in one of nine 15-chromosome plants having *lata* or *semilata* characters "in which the somatic chromosomes at metaphase were more or less completely divided transversely into two." They refer to Agar's studies of this phenomenon in Lepidosiren in which it was found that the chromatin matter only, and not the linin of the chromosomes, segmented. Their assertion that certain somatic chromosomes of one of their 15-chromosome plants were "more or less completely divided transversely into two," fails to give the reader a precise idea of the conditions which they observed. By "more or less completely" do they mean that some were completely divided into separate parts and others only partially so? Their meaning is obscure.

During the year spent in Professor Grégoire's laboratory especial attention was given to the study of these chromosomes and ample evidence was found to indicate that these supposed segments, at least in the majority of cases, were merely whole chromosomes with clear spaces, or unstained regions. As Gates and Miss Thomas state, these light areas were sometimes observed near the middle, and sometimes nearer one end (figs. 7, 8, 9, 10). Often, also, two such areas were found in one chromosome, giving the body the appearance of having divided transversely into three parts (fig. 10*c*). Frequently, clear spaces were found in two or more chromosomes of a group (fig. 9), but I recall no instance in which all displayed this peculiarity. In heavily stained material it is often possible to detect the delicate side lines connecting these supposed segments of chromosomes, thus proving that in these particular cases at least, we are dealing with whole chromosomes (figs. 10*a*, 10*b*, 12, 13). In extracting the stain (iron-haematoxylin) from material designed for chromosome studies the color is usually removed entirely from the linin; as a result, it is impossible to detect the linin connections between the two heavily stained portions of the chromosome (*i. e.*, the linin surrounding the unstained or clear area) and the observer is led to conclude that actual and complete transverse segmentation has occurred. However, if one examines these segments in somatic metaphase, it will be apparent, in the majority of cases at least, that these supposed segments lie in line with each other. (See figs. 7, 8, 9.) If the observer will connect the supposed segments with delicate side-lines and will then fill in the inclosed clear areas with soft crayon, he will usually find that he has a perfectly normal-appearing whole chromosome and that this is true whether the supposed segments are curved or straight. One occasionally finds these darkly stained portions entirely disconnected and out of line with each other (fig. 11), but there is generally plenty of evidence to show that they have been roughly torn apart by the knife in sectioning. Nuclear prophase chromosomes with clear areas are very frequently encountered (fig. 6). If such bodies are indeed whole chromosomes and not segments of chromosomes, then we should find corresponding halves of such chromosomes in anaphase, and these are shown in figs. 12 and 13.

These clear areas may indicate points at which the chromatin matter has undergone some change whereby it becomes incapable of taking the stain, or of retaining it, when the section is extracted for chromosome study; it is also possible that these regions are empty spaces resulting from shrinkage of the chromatin matter following fixation. The fact that chromosomes are occasionally torn apart through this region in sectioning, is certainly indicative of weakness at this point.

The above facts are related merely to show that owing to the complete extraction of stain from the linin surrounding clear areas in chromosomes, one may be led to conclude that a chromosome has actually divided transversely into two parts when it has not. However, the writer has not attempted to prove that chromosomes never break apart through these regions, for it is possible that they do, in rare instances.

In *Oenothera*, so far as I am aware, chromosomes with clear areas have been found in somatic tissues only. Geerts and Gates and Miss Thomas do not mention having observed this peculiarity in any of the chromosomes of the generative cells of 14+-chromosome forms which they studied. Furthermore, if we regard the appearance of these unstained regions in somatic chromosomes merely as a stage preceding fragmentation, then we should find numerous instances of fragmentation in groups of somatic chromosomes, but we do not.

While the plant in which Gates and Miss Thomas found somatic chromosomes with clear areas chanced to be a 15-chromosome mutant, it should not be inferred that this peculiarity is characteristic of 15-chromosome forms only, nor even of 14+-chromosome forms only. *It is not associated with the 14+-chromosome condition in* Oenothera, *for these chromosomes are just as common in 14-chromosome* O. Lamarckiana *and other 14-chromosome forms as in 14+-chromosome individuals.* I have found plants (mutants and hybrids) in the *Lamarckiana* group with the following somatic chromosome numbers; 14, 14^{+1}, 15, 16, 18, 19, 21, 22, 23, 24, 26, 28, 29 and 30—the number being constant, so far as ascertained, for each type and for each individual of the type—and have observed these chromosomes with clear spaces in every type studied. It was found that this peculiarity was no more common in hybrids and in mutants than in *O. Lamarckiana*, nor more common in 14+- than in 14-chromosome forms.

Since each of the three 14^{+1}-chromosome plants had a 15-chromosome mother (♀ *O. lata* × ♂ *O. Lamarckiana*) it is probable that the small chromosome was derived from the mother, and that each individual was the product of ♀ 7^{+1} + ♂ 7. However, Gates and Miss Thomas found that the extra chromosome of 15-chromosome forms degenerated only occasionally; furthermore, since they found chromosomes pulling apart in the middle and sometimes "fragmenting into three parts" in 14- as well as in 15-chromosome plants, it is conceivable that *O. lata* × *O. Lamarckiana* might produce a 14^{+1}-chromosome offspring by means of a ♀ 8 + ♂6^{+1} union. If the small body of de Vries's *rubrinervis* was merely a piece which had become detached from one of the ordinary chromosomes, then it may have been derived either from the 15-chromosome mother or the 14-chromosome father (♀ 7^{+1} + ♂ 7 or ♀ 7 + ♂ 7^{+1}). The small body may have been derived from the 14-chromosome parent by still another means. Since the heterotypic chromosomes of *O. Lamarckiana* are occasionally distributed in groups of 6 and 8, it is quite possible that one of the members of the 8-chromosome group sometimes fragments and degenerates just as it does at times in the 8-chromosome groups of 15-chromosome forms.

Should a fragment of the 8th chromosome chance to persist, a 7^{+1}-pollen grain might be formed.[8]

Thus far, no 14^{+1}-chromosome offspring of *O. Lamarckiana* has been reported; hence it is probable that the small chromosome of the three 14^{+1}-chromosome mutants was derived from the *lata* mother in each case. Whatever its origin, as a somatic body, it is capable of dividing longitudinally and of performing other normal activities of ordinary chromosomes during cell division.

4. *Fate of the Small Chromosome in Succeeding Generations*

During the years in which this work was conducted at the Station for Experimental Evolution, Dr. G. H. Shull kindly gave me the privilege of studying all forms of interest which appeared in his cultures of *Oenothera*, and it was among his *lata* × *Lamarckiana* hybrids that No. 3878 appeared in 1908. With perseverance I succeeded in obtaining a few seeds from this plant, selfed, and the offspring were grown by Dr. Shull in 1909. However, since the somatic chromosome number of the parent was not determined until late autumn of that year, the importance of determining the chromosome numbers of the offspring was not appreciated until the season had passed, consequently no fixations were prepared and no records of the vegetative characters of these plants are in my possession. The fate of the small chromosome is, therefore, unknown.

Did a portion or all of the offspring of No. 3878, selfed, have 14^{+2}-chromosomes? Since Geerts found that 7 whole (male and female) chromosomes of 21-chromosome forms may be eliminated and Gates and Miss Thomas have shown that 1 or more of the ordinary (male) chromosomes of 15-chromosome forms may fragment and degenerate, it is probable that the small chromosome of 14^{+1}-chromosome forms is eliminated in a similar manner, in many cases, at least. If a portion of the male and female gametes had 7^{+1}-chromosomes and the remainder 7, then it is possible that 14-, 14^{+1}- and 14^{+2}-chromosome forms were produced.[9] We know that 14-, 15- and 16-chromosome forms never have duplicate vegetative characters; would the same be true of 14-, 14^{+1}- and 14^{+2}-chromosome plants?

---

[8] Likewise, it is possible that 7^{+1}-chromosome female gametes are occasionally produced in this manner by 14-chromosome forms. All facts considered, it is more probable that 7^{+1}-chromosome female gametes, capable of functioning, than that 7^{+1}-chromosome male gametes, capable of functioning, are produced by *O. Lamarckiana* and other 14- or 15-chromosome forms.

[9] If 7^{+1}-chromosome gametes are produced by 14^{+1}-chromosome forms, it is probable that they are unisexual.

5. *Chromosomal Individuality*

Gates found that the chromosomes of *Oenothera* present no constant differences in size and shape, and, with the exception of the small chromosome in the 14^{+1}-chromosome mutants, the observations of the writer are in full accord with those of Gates. The small member of the 14^{+1}-chromosome group, however, presents a very conspicuous exception, for we have seen that *it may be recognized constantly by its shape and by its diminutive size.* It furnishes very strong evidence in support of the theory of chromosomal individuality.

6. *O. rubrinervis*

While de Vries has shown that *rubrinervis* is one of the common mutant types produced by *O. Lamarckiana*, I have found no mutant in the Long Island or Indiana cultures of *O. Lamarckiana* × *O. Lamarckiana* which could be said to duplicate the type which de Vries has figured and described ('09, Vol. I, figs. 49 and 67). However, a 14-chromosome mutant (type 3514, see page 508) which seems to be a modified form of *O. rubrinervis* de Vries, is very common. It differs from the Amsterdam plant chiefly in height and branching habits, attaining the full height of *O. Lamarckiana*, and, like the latter form, giving off a number of long rosette branches. It is shown as an early flowering plant in text figure 6 and as a late flowering plant in text figure 7. MacDougal's excellent photograph ('05, Pl. XXI) of "*O. rubrinervis*" at the New York Botanical Garden portrays the branching habits of the Cold Spring Harbor mutant very clearly. Since it appears to duplicate the vegetative characters of the C.S.H. plant, it is probable that it also had 14 chromosomes. In response to an inquiry concerning the history of this individual, Dr. MacDougal stated that the plant photographed had been grown directly from seeds received from Professor de Vries.

Type 3514 produced quadrangular buds with deep red sepals; the flowers also were deeper yellow than *Lamarckiana* flowers. Large quantities of seemingly good pollen were exposed, consisting of 3-lobed grains, and seeds from these plants, selfed, were obtained without difficulty.

In the spring of 1913 Professor de Vries very kindly provided me with seeds of the Amsterdam mutant. These were sown in March of that year, but owing to drought, late transplantation and a destructive attack of aphids, which showed a curious preference for

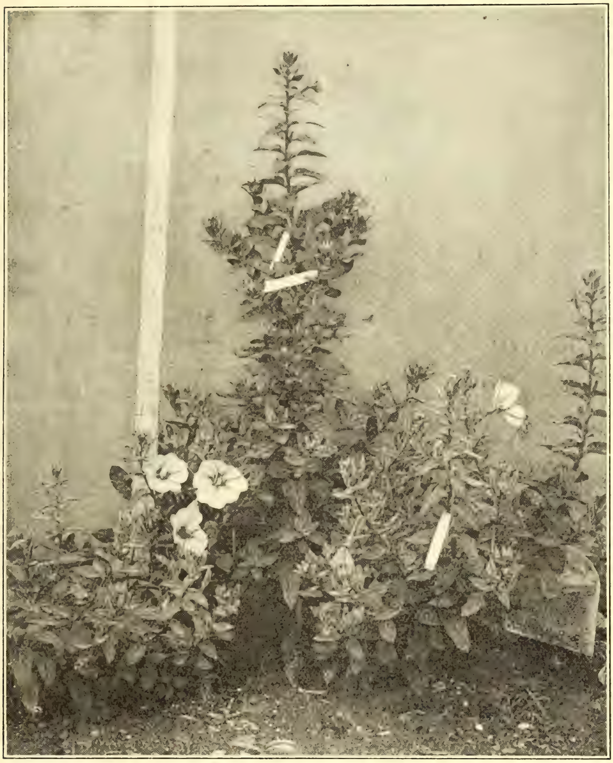

FIG. 6. Type 3514, plant No. 3514, C. S. H., 1908. Mutant offspring of *O. Lamarckiana*. Supposed to be a modified form of de Vries's *rubrinervis*. Photographed during early flowering period.

rubrinervis, only one plant came to flower. This individual corresponded perfectly with de Vries's descriptions and photographs of *rubrinervis*, but did not duplicate the C.S.H. type, though it had many characters in common with it. Their resemblance was noted in the

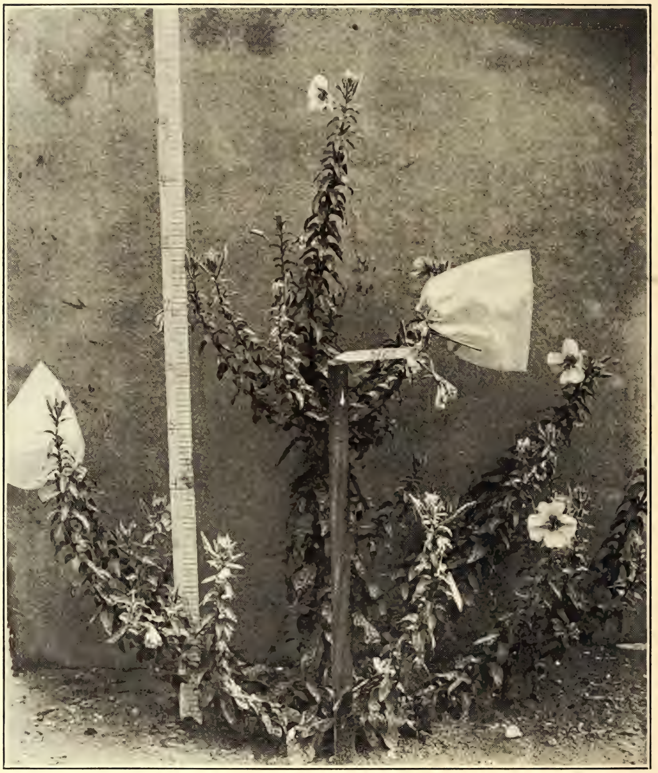

FIG. 7. Type 3514, plant No. 3464, 1908. Mutant offspring of *O. Lamarckiana*. Late flowering period.

striking similarity of the young rosettes, in the red sepals, deep yellow petals, quantities of seemingly good pollen produced, long seed-capsules, color and shape of the leaves. They differed chiefly in height and branching habits. If the reader will compare the C.S.H. and New York Botanical garden type with de Vries's photographs, their dissimilarity with respect to the latter character will be apparent at once. The C.S.H. mutant is found in cultures of *O. Lamarckiana* × *O. Lamarckiana* (also among offspring of *O. lata* × *O. Lamarckiana* and *O. lata*, selfed), while it is quite possible that de Vries's *rubrinervis* is produced by selfed *Lamarckiana.* If one is inclined to believe that de Vries's *rubrinervis* is the product of inbred *Lamarckiana* and the C.S.H. type the result of crossing one *Lamarckiana* individual with another, unrelated *Lamarckiana*, one should not overlook the fact that de Vries also obtained the Amsterdam type from *O. lata* × *O. nanella*, *O. lata* × *O. brevistylis*, *O. nanella* × *O. brevistylis*, *O. scintillans* × *O. nanella*, *O. lata*, *O. oblonga*, etc.

Since Gates found 14 chromosomes in *rubrinervis* and since the plants in which he made these counts were also grown in America, it would be instructive to learn whether they duplicated the vegetative characters of de Vries's form throughout, or whether they also were modified individuals of the same type as the New York Botanical Garden and Cold Spring Harbor mutants. Had the small chromosome been present in the somatic cells of the plant which Gates studied as it was in the Amsterdam mutant, it is not probable that it would have been overlooked. It will be borne in mind, of course, that it has not yet been shown that all plants having the same type of vegetative characters as de Vries's mutant have 14^{+1} chromosomes; for, thus far, the somatic chromosome number of but one Amsterdam *rubrinervis* has been reported. It may be that the small remnant merely chanced to be handed down from the *lata* mother of No. 6082 without affecting the characters of the *rubrinervis* offspring.

SUMMARY

1. The primary object of this series of three papers, of which the present report is the first, is to discuss, in the light of the Cold Spring Harbor and Louvain studies of somatic chromosome number in *Oenothera Lamarckiana* and its derivatives, certain theories and conclusions which Gates has advanced from time to time, and which Gates and Miss Thomas have based upon the results of their investigations.

2. Five hitherto undescribed types of 14-chromosome mutants have been recognized in Cold Spring Harbor cultures of *O. Lamarckiana*, *O. lata*, *O. nanella*, etc.; namely, types 2787, 2803, 3539, *O. plicatula* and *O. delicatula*. In addition to the foregoing, 14 chromosomes have been counted in a type (3514) figured and described by MacDougal in 1905 as *O. rubrinervis*. This form is believed by the writer to be a modified form of the Amsterdam mutant. It is not known whether the *rubrinervis* in which Gates counted 14 chromosomes (reported in 1908) duplicated the characters of the Dutch, or of the American type.

3. One plant was found in a 1908, and another in a 1909, culture of *O. lata* × *O. Lamarckiana*, each having 14 chromosomes of the usual size and 1 very small one. The two plants had the same type of vegetative character and the mutant form which they represented will be known as *O. aberrans*. The same condition was found in a *rubrinervis* mutant from one of de Vries's 1912 cultures of *O. lata* × *O. Lamarckiana*.

4. In each of the three plants the small chromosome usually, though not invariably, occupied a peripheral position on the equatorial plate and was commonly alloted the full space of an ordinary chromosome.

5. The small chromosome of de Vries's *rubrinervis* differed slightly in appearance from that of *O. aberrans* and the somatic characters of the two forms were strongly contrasted.

6. It was not ascertained in any one of the three plants whether or not the small chromosome invariably accompanied the 14 ordinary chromosomes in the somatic cells examined, but it was clearly demonstrated that it was, at least, seldom absent.

7. Gates and Miss Thomas found that one of the 15 chromosomes of *O. lata* may sometimes fragment and degenerate during male reduction and have presented evidence which indicates that male gametes may be formed containing one or more fragments of chromosomes in addition to the ordinary, whole chromosomes. We have no evidence to show whether 7^{+1}-chromosome male gametes, *capable of functioning*, are produced by any form. However, it is probable that the extra chromosome of 15-chromosome forms sometimes fragments during female reduction and that 7^{+1}-chromosome eggs, capable of functioning, are produced occasionally.

8. Since each of the three 14^{+1}-chromosome mutants was derived from 15-chromosome *O. lata* × 14-chromosome *O. Lamarckiana*, it is probable that all were products of ♀ 7^{+1} + ♂ 7.

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EXPLANATION OF PLATE XXIV.

All figures were drawn with the aid of a camera lucida, Bausch and Lomb 1 inch ocular and oil immersion, ap. 1.9. Figures drawn from sections of root-tips taken from greenhouse rosettes. Cut 7 μ thick and stained with Heidenhain's iron-alum haematoxylin. Figures 1–11 taken from transverse, and 12–13 from longitudinal, sections.

FIG. 1. *O. aberrans,* plant No. 3878, C. S. H., 1908. Mutant offspring of *O. lata* × *O. Lamarckiana.* Polar view of metaphase group showing 14 chromosomes of the usual size and 1 very small one, the latter occupying a peripheral position on the equatorial plate.

FIG. 2. *O. aberrans,* plant No. 4605, C. S. H., 1909. Mutant offspring of *O. lata* × *O. Lamarckiana.* Polar view of metaphase group showing 14^{+1}-chromosomes, the small body lying farther in the group than in the preceding figure.

FIG. 3. *O. aberrans,* plant No. 4605, C. S. H., 1909. Polar view of metaphase group showing 14^{+1}-chromosomes, the small body occupying a peripheral position on the equatorial plate.

FIG. 4. *O. rubrinervis,* plant No. 6082, Amsterdam, 1912. Mutant offspring of *O. lata* × *O. Lamarckiana.* Polar view of metaphase group showing 14^{+1}-chromosomes.

FIG. 5. *O. rubrinervis,* plant No. 6082, Amsterdam, 1912. Same as fig. 4. The small chromosome of figs. 4 and 5 occupies a peripheral position on the equatorial plate in each case. Note also that the small body of this plant appears to be almost round and apparently somewhat broader than the ordinary chromosomes.

FIG. 6. *O. gigas,* plant No. 3676, C. S. H., 1908. Offspring of *O. gigas.* Nuclear prophase stage showing chromosome *a* with clear area near one end.

FIG. 7. *O. Lamarckiana,* plant No. 970, C. S. H., 1907. Offspring of *O. Lamarckiana.* Polar view of metaphase group of 14 chromosomes showing *a* with clear area at the middle of the body.

FIG. 8. *O. Lamarckiana,* plant No. 983, C. S. H., 1907. Offspring of *O. Lamarckiana.* Polar view of metaphase group of 14 chromosomes showing *a* with clear area between middle and extremity of the body.

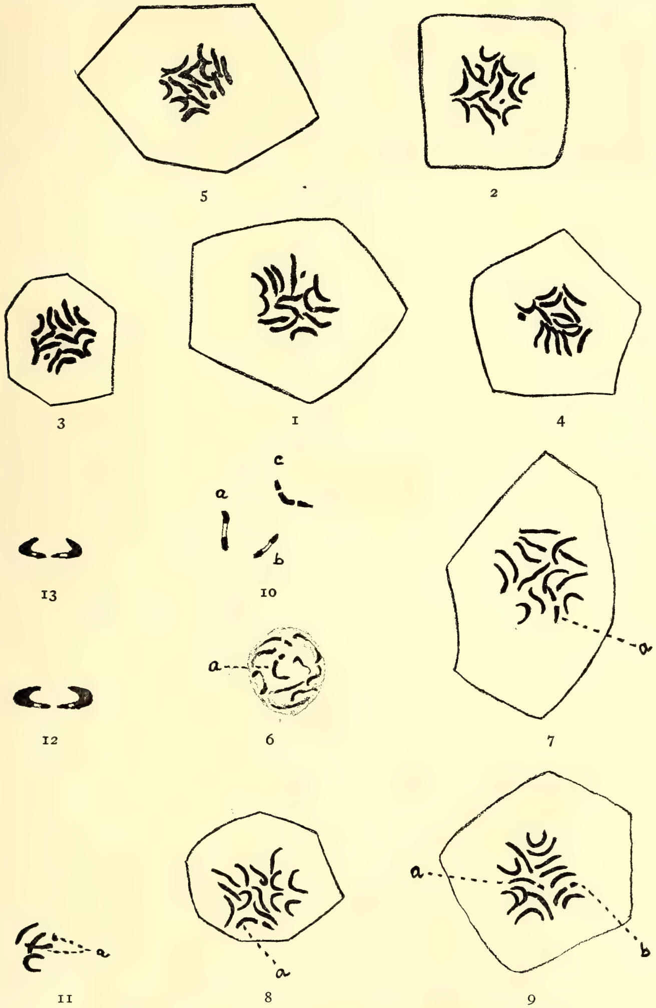
Fig. 9. *O. Lamarckiana*, plant No. 2488, C. S. H., 1908. Offspring of *O. lata* × *O. Lamarckiana.* Polar view of metaphase group of 14 chromosomes showing *a* and *b* each with a clear area near one extremity of the body.

Fig. 10. Chromosome *a*, plant No. 3433, C. S. H., 1908; a 21-chromosome mutant offspring of *O. lata* × *O. Lamarckiana.* Chromosome *b*, plant No. 3627, C. S. H., 1908; *O. albida*, a 15-chromosome mutant offspring of *O. Lamarckiana.* Chromosome *c*, plant No. 1133, 1907; *O. gigas* offspring of *O. gigas.* All taken from polar views of metaphase groups; *a* and *b* each display a single large, clear area, while two such regions may be observed in *c*. Owing to the fact that *a* and *b* are more heavily stained than *c* and than the chromosomes of figs. 6–9, the linin surrounding the clear area in *a* and *b* appears as delicate side-lines connecting the heavily stained portions, while it is invisible in the remaining chromosomes mentioned. The relative positions of the heavily stained regions clearly show that we are dealing with whole chromosomes and not with fragments.

Fig. 11. Type 5509, plant No. 5509. C. S. H., 1908. Supposed to be a modified form of *O. oblonga.* Mutant offspring of *O. Lamarckiana.* Polar view of a metaphase group showing chromosome *a* torn apart in the region of the clear area and the small extremity displaced, probably by the knife in sectioning.

Fig. 12. *O. nanella*, plant No. 2788, C. S. H., 1908. Offspring of *O. nanella.* Lateral view of daughter chromosomes in anaphase showing clear areas in corresponding regions. Note the delicate linin side-lines connecting the heavily stained portions.

Fig. 13. *O. gigas*, plant No. 4662, C. S. H., 1909. Offspring of *O. gigas.* Description the same as for fig. 12.


5
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c
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10
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6
7
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a
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9

Lutz: Diminutive Chromosomes in Oenothera.

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THE UREDINALES FOUND UPON THE ONAGRACEAE

G. R. BISBY

The members of the family Onagraceae, while of cosmopolitan distribution, are particularly American. Western North America is especially rich in species belonging to this family. Similarly, while a few rusts upon members of the Onagraceae are scattered over many parts of the world, the majority of them are known only from America. The Sydows, in the three published volumes of their Monograph of the Uredinales, list 27 species of rusts upon the Onagraceae. Of these, 15 species are given as existing only in North America, 3 species only in South America, and 2 species both in America and in other countries. Of the species for which the Americas are not included as localities, 3 have been found to be present. Thus, of the 27 species listed by the Sydows, 23 occur in the Western Hemisphere, and 21 of these have been found in North America. In addition to the species published in the Sydows' monograph, there are *Aecidium Anograe* and *Puccinia Fuchsiae*, both known only from North America. *Puccinia Veratri*, a widely distributed species, has rather recently been found to have its aecial stage upon certain of the Onagraceae; the telial stage only is listed in Sydow, and not included above. *Puccinia Nesaeae* (Ger.) Ell. & Ev., listed in Sydow as occurring upon Nesaea, is misplaced, the host in reality being Ludwigia, a genus of the Onagraceae.

The family Onagraceae has proved perplexing to the phanerogamic taxonomists; a glance at the lack of uniformity in ideas of nomenclature and arrangement of species as represented in various floras attests to the uncertainty of specific characters in this group of plants. These very uncertainties and variabilities have given this family important consideration from an evolutionary standpoint. An extensive literature has grown around the genus Oenothera alone.

[The **Journal** for November (3: 481–526) was issued Dec. 23, 1916.]

So, too, the rusts occurring upon these hosts have been found to be variable, uncertain, and confusing. Arthur[1] has dealt with similar conditions in the case of the rose rusts, in which the variability of the host appears to be reflected in a similar degree by the variation in the rust. In his discussion of this condition Arthur states (p. 28) that "each species of Phragmidium has attained a degree of orthogenetic development and a diversity of characters corresponding to the hosts on which it occurs, always, however, with a certain lag due to the inhibiting nature of parasitism." It would seem that parallel conditions as regards variability, both with the hosts and their rusts, obtain in both the Rosaceae and Onagraceae.

Considerable work has been done upon the rusts of the Onagraceae; the results, however, are rather scattered and somewhat conflicting, and the species described have seemed often to be of doubtful validity. The Sydows, as previously suggested, have done much toward systematizing the knowledge of these rusts; Holway has published valuable descriptions and notes on the species of Puccinia occurring upon this group. Descriptions of some of these rusts have already appeared in the North American Flora. Many other writers have added their ideas; yet it was apparent that further work upon a considerable number of collections of some of these species of rust should result in an attainment of greater order. The facilities of the Arthur herbarium have afforded to the writer an unequaled opportunity to study a large number of collections; preparation of manuscript upon the rusts for the North American Flora rendered it imperative that additional critical studies be made of Onagraceous rusts.

The ideas regarding relationship, correlation, and classification, advocated in this paper, should be considered but the elaboration of the opinions held by Dr. J. C. Arthur, and by the workers in his laboratory. It has been possible to bring together the data presented, through the courtesy of Dr. Arthur in placing the facilities of the laboratory at the writer's disposal. Furthermore, thanks are due to Messrs. F. D. Kern, C. R. Orton, C. A. Ludwig, and especially to Prof. H. S. Jackson, for much assistance.

The term "correlation," as used in this paper and by other writers from this laboratory, is intended to express an idea of relationship of rusts based on similarities of morphological characters. This similarity may be expressed in different ways. One of the earliest noted resemblances between species was that between certain Uromyces and Puccinia forms with the same life cycle, the only difference being in the possession, by the one species, of one-celled teliospores, by the other of two-celled teliospores. Fries[2] noted the fact that such an analogy sometimes existed. Orton[3] has reviewed the literature pertaining to this type of correlation, and shown some specific instances. This kind of correlation is shown in the Onagraceae rusts by the resemblance of *Uromyces plumbarius* to *Puccinia Epilobii-tetragoni.*

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[1] Arthur, J. C., North American Rose Rusts. Torreya 9: 21–28. 1909.

A second type of correlation is that illustrated by a similarity in the characters of a short-cycled species and a long-cycled heteroecious species bearing aecia upon the telial host of the short-cycled rust. Dietel,[4] and about the same time, Fischer,[5] pointed out this type of resemblance. Travelbee,[6] from this laboratory, has briefly summarized the literature regarding this type of correlation, and listed some proven examples. This indicated relation has been successfully used to predict alternate hosts: indeed, in the Onagraceae rusts, through the similarity of the telial stage of *Puccinia Epilobii* to that of *Puccinia Veratri*, the alternate host of the latter was forecasted, as is noted later in this article.

A third type of correlation is that between two species of rust, with life cycles of different lengths, occurring upon the same or similar hosts. Fischer[7] has indicated such a relation between two species of rust upon Epilobium; other possibilities are suggested in this paper.

It is obvious that a similarity of morphological characters does not of necessity reflect a phylogenetic relationship. Certainly, however, when such similarities are found between rusts upon related hosts, it is a noteworthy suggestion of a definite relationship between the rusts, and it seems to the writer that relationships may be inferred in certain cases even when there is some slight variation between the parallel characters of two such species of rust.

Races are designated in this paper as occurring within the long-cycled autoecious species discussed. The idea embodied is somewhat different from that of physiological or biological races. The latter designation of races is used, in the Uredinales, in cases where cultures have shown that there are certain restrictions upon the transference of a rust from host to host. The idea made use of here is that of morphological races—races separated upon the same ideas of differentiation upon which species are ordinarily based, but the differences being not of sufficient value nor constancy to make possible a separation into species. In the absence of cultural data, these races are suggested for convenience. The idea is somewhat that of varieties under a species. The word variety is not used, however, as that would result in a cumbersome, and perhaps inaccurate, nomenclature; a tentative division into morphological races seems to afford an opportunity to systematize the arrangement of specimens representing rather variable species.

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[2] Fries, E. M., Summ. Veg. Scand. 1: 514. 1846.

[3] Orton, C. R., Correlation between certain species of Puccinia and Uromyces. Mycol. 4: 194–204. 1912.

[4] Dietel, P., Uredinales, in Engler and Prantl, Pflanzenfam. 1^{1++}: 69. 1897.

[5] Fischer, Ed., Beitr. Krypt. Schweiz 1^1: 109. 1898.

[6] Travelbee, H. C., Correlation of certain long-cycled and short-cycled rusts. Proc. Ind. Acad. Sci. 1914: 231–234. 1915.

[7] Fischer, Ed., Beitr. Krypt. Schweiz 2^2: 154–155. 1904.

A perfectly consistent treatment of the evening primrose rusts, embracing all the species, is now impossible. This study is therefore made primarily in an attempt to draw attention to some of the questions demanding answer, and in the hope that collections, cultures, and studies may eventually be made to clarify and arrange our knowledge of this interesting group of rusts.

The main points brought out in this paper are: the grouping together of the long-cycled autoecious forms of Puccinia upon the Onagraceae into one species, and the considerations involved; some notes upon the heteroecious forms which include the Onagraceae in their life cycle; some correlations indicated between different species and races; keys to aid in the diagnosis of the various rusts in question. Several incidental points are discussed. Species not known in North America are dealt with only briefly. Descriptions of several of the species under discussion are not added here, since that would appear to be an unnecessary duplication. References to descriptions easily available are given in such cases.

The keys herewith presented offer difficulties at some points, due partly, as pointed out above, to the fact that correlated species possess spore-forms morphologically indistinguishable. The different considerations, however, usually can be utilized to place a specimen. The abbreviations N.A. (North America), S.A. (South America), Eur. (Europe), etc., and the symbols O (pycnia), I (aecia), II (uredinia), and III (telia), used in places, are for brevity.

Key, based upon life history

Pycnia and aecia only occurring upon Onagraceae.	
Aecia diffused.	*Puccinia Veratri.*
Aecia in groups.	
Aeciospore wall thick, 2–3 μ.	*Aecidium Anograe.*
Aeciospore wall thin, 1 μ.	
Spores larger, 14–21 μ long.	*Puccinia Peckii.*
Spores smaller, 13–15 μ long (Eur.)	*Aecidium Circaeae.*
Pycnia, aecia, and telia occurring upon Onagraceae.	
Teliospores up to 60 μ in length.	*Puccinia Jussiaeae* (*Puccinia Ludwigiae*).
Teliospores up to 45 μ in length (Eur.)	*Puccinia Epilobii-Fleischeri.*
Pycnia, aecia, uredinia, and telia occurring upon Onagraceae.	
Teliospores one-celled.	*Uromyces plumbarius.*
Teliospores two-celled.	*Puccinia Epilobii-tetragoni.*
Characters of the latter (S. A.)	*Puccinia luxurians.*
Life-history uncertain; possibly same as *P. Epilobii-tetragoni* (Afr.)	*Puccinia Krookii.*
Uredinia and telia only occurring upon Onagraceae.	
Telia within or below epidermis.	*Pucciniastrum pustulatum.*
Characters similar to the latter (Eur.).	*Pucciniastrum Circaeae.*
Pycnia (when formed) and telia occurring upon Onagraceae.	
Teliospore apex scarcely thickened, 1.5–4 μ.	
Teliospore wall smooth, 3 μ thick.	*Puccinia sphaeroidea?**
Teliospore wall verrucose, 1.5–2 μ thick.	
Teliospores smaller, 13–18 by 27–37 μ.	*Puccinia scandica.*
Teliospores larger, 17–25 by 30–44 μ.	*Puccinia Epilobii.*
Teliospore apex considerably thickened, 5–12 μ.	
Teliospores somewhat smaller, up to 40 μ.	*Puccinia Circaeae.*
Teliospores medium, up to 50 μ.	*Puccinia Fuchsiae.*
Teliospores somewhat larger, up to 60 μ.	*Puccinia gigantea.*
Uredinia only known upon Onagraceae (S. A.).	*Uredo oenothericola.*

Key, based upon spore forms independently

Aecia in groups, *i. e.*, infection local.	
Aecisopore wall thick, 2–3 μ.	*Aecidium Anograe.*
Aeciospore wall thin, 1 μ.	
Aecia small, 0.2–0.3 mm. across.	
Aeciospores 15–21 μ long.	*Puccinia Jussiaeae* (*Puccinia Ludwigiae*).
Aeciospores 13–15 μ long.	*Aecidium Circaeae.*
Aecia larger, 0.3–0.6 mm. across.	*Puccinia Peckii.*

* Doubtfully upon Onagraceae.

Aecia diffused, *i. e.*, infection general.	
Morphological characters similar; correlated species; aeciospores usually only to 20–21μ in length.	*Uromyces plumbarius*, *Puccinia Epilobii-tetragoni*, *Puccinia Epilobii-Fleischeri*
Similar to above, except aeciospores often up to 23–24 μ.	*Puccinia Veratri.*
Uredinia without peridium; urediniospore-wall colored.	
Uredinia often gregarious on spots.	*Uredo oenothericola.*
Uredinia scattered; spots none; correlated.	*Uromyces plumbarius*, *Puccinia Epilobii-tetragoni*, (*Puccinia luxurians*, *P. Krookii*).
Uredinia with peridium; urediniospore-wall colorless.	
Characters similar.	*Pucciniastrum pustulatum*, *Pucciniastrum Circaeae.*
Telia within or below epidermis.	
Characters similar.	*Pucciniastrum pustulatum*, *Pucciniastrum Circaeae.*
Telia erumpent.	
Teliospores one-celled.	*Uromyces plumbarius.*
Teliospores two-celled.	
Teliospore apex considerably thickened, 4–12μ.	
Telia scattered, not upon spots.	
Characters similar; correlated.	*Puccinia Epilobii-tetragoni*, *Puccinia Epilobii-Fleischeri.*
Characters apparently those of *Puccinia Epilobii-tetragoni.*	(*Puccinia luxurians* *Puccinia Krookii*).
Telia gregarious, upon spots.	
Teliospores smaller, to 40 μ long.	*Puccinia Circaeae.*
Teliospores to 50 μ long.	*Puccinia Fuchsiae.*
Teliospores larger, to 60 μ long.	
Wall cinnamon-brown.	*Puccinia Jussiaeae* (*Puccinia Ludwigiae*).
Wall paler, especially below.	*Puccinia gigantea.*
Teliospore apex little thickened, 1.5–4 μ.	
Teliospore wall smooth, 3 μ thick.	?*Puccinia sphaeroidea.*
Teliospore wall verrucose, 1.5–2 μ thick.	
Teliospores comparatively smaller, 13–18 by 27–37 μ.	*Puccinia scandica.*
Teliospores comparatively larger, 17–25 by 30–44 μ.	*Puccinia Epilobii.*

KEY TO SPECIES AND RACES, BASED UPON HOST AND INCLUDING GEOGRAPHIC DISTRIBUTION

Gayophytum; N. A., S. A.; O, I, II, III, *Puccinia Gayophyti* race of *Puccinia Epilobii-tetragoni.*

Chamaenerion
Western N. A.; Eur.; O, I, II, III;
III spores to 40 μ, I spores usually to 20 μ.
Puccinia Gayophyti race of *Puccinia Epilobii-tetragoni.*
N. A.; Eur.; O & I
I often to 24 μ. *Puccinia Veratri.*
N. A.; Eur.; II & III only;
III spores often within epidermis. *Pucciniastrum pustulatum.*
Western N. A.; Eur.; III only;
III spores to 60 μ. *Puccinia gigantea.*
Epilobium
Teliospore apex thickened.
As first three under *Chamaenerion.*
Puccinia Gayophyti race of *Puccinia Epilobii-tetragoni,*
Puccinia Veratri,
Pucciniastrum pustulatum.
Eur.; O, I, III. *Puccinia Epilobii-Fleischeri.*
Afr.; uncertain. *Puccinia Krookii.*
Teliospore apex little thickened
N. A.; Eur.; III only,
III spores 13–18 by 27–37 μ. *Puccinia scandica.*
III spores 17–25 by 30–44 μ. *Puccinia Epilobii.*
Boisduvalia glabella only; Western N. A.; (O, I), II,
III................*Puccinia glabella* race of *Puccinia Epilobii-tetragoni.*
Taraxia; Western N. A.; O, I, II, III
Puccinia heterantha race of *Puccinia Epilobii-tetragoni.*
Boisduvalia (*P. Boiduvaliae*)
Chylisma (*P. Oenotherae*)
Eulobus (*P. Eulobi*)
Clarkia
Godetia (*P. Clarkiae*)
Phaeostoma
Sphaerostigma (*P. Sphaerostigmatis*)
Zauchneria (*P. Zauchneriae*)
O, I, II, III; Western N. A.; *Puccinia Oenotherae* race of *Puccinia Epilobii-tetragoni.*
Gaura
N. A.; O, I, II, III; aecia diffused *Uromyces gaurinus* race of *Uromyces plumbarius.*
N. A.; O, I; aecia grouped. *Puccinia Peckii.*
Kneiffia; N. A., O, I, II, III.
Uromyces Oenotherae race of *Uromyces plumbarius.*
Oenothera
N. A.; O, I, grouped. *Puccinia Peckii.*
N. A.; O, I, II, III; aecia diffused.
III spores one-celled.
Uromyces Oenotherae race of *Uromyces plumbarius.*
III spores two-celled.
?*Puccinia Oenotherae* race of *Puccinia Epilobii-tetragoni.*

S. A.; O, I, II, III (?).	*Puccinia luxurians.*
S. A.; II only known.	*Uredo oenothericola.*
Luxavia; N. A.; O, I, II, III.	*Uromyces plumbarius* race of *Uromyces plumbarius.*
Pachylopus	
N. A.; O, I, II, III; aecia diffused	*Uromyces plumbarius* race of *Uromyces plumbarius.*
N. A.; O, I; aecia grouped.	*Puccinia Peckii.*
Onagra	
N. A.; O, I, II, III; aecia diffused	*Uromyces plumbarius* race of *Uromyces plumbarius.*
N. A.; O. I; aecia grouped.	*Puccinia Peckii.*
Megapterum, N. A.; O, I, II, III	*Uromyces Fremontii* race of *Uromyces plumbarius.*
Meriolix, N. A.; O. I.	*Puccinia Peckii.*
Circaea	
N. A.; Eur.; III; III erumpent	*Puccinia Circaeae.*
Eur.; II, III; III not erumpent.	*Pucciniastrum Circaeae.*
Eur.; O, I.	*Aecidium Circaeae.*
Anogra, central U. S.; O, I.	*Aecidium Anograe.*
Jussiaea	
N. A.; S. A.; I, III; spores up to 54 μ long.	*Puccinia Jussiaeae* (*Puccinia Ludwigiae*).
Western N. A.; III only; spores up to 32 μ long......	?*Puccinia sphaeroidea.*
Ludwigia, N. A.; O, I, III.	*Puccinia Jussiaeae* (*Puccinia Ludwigiae*).
Fuchsia, Mexico; III only.	*Puccinia Fuchsiae.*

1. Aecidium Anograe Arthur, Bull. Torrey Club **28**: 664. 1901.

O. Pycnia amphigenous, grouped on the spots with the aecia, inconspicuous, subepidermal, honey-yellow becoming brownish, globose, 100–120 μ in diameter by 80–100 μ in height; ostiolar filaments 30–80 μ long.

I. Aecia amphigenous, chiefly hypophyllous, gregarious on roundish or irregular reddened spots, cylindrical, 0.2–0.3 mm. in diameter by 0.5–0.6 mm. in height; peridium white, margin erect, toothed; peridial cells rectangular, 18–24 by 22–35 μ, slightly overlapping, the outer wall 6–10 μ, striate, the inner wall 3–5 μ, coarsely verrucose; aeciospores irregularly globoid or ellipsoid, 18–23 by 22–26 μ; wall pale yellow, thick, 2–3 μ, evenly verrucose.

On Onagraceae: *Anogra pallida* (Lindl.) Britt. (*Oenothera pallida* Lindl., *Anogra Vreelandii* Rydb.) Nebraska.

Type Locality: Long Pine, Nebraska, on *Anogra pallida.*

Distribution: Known only from the dry northwestern part of Nebraska.

Exsiccati: Barth., Fungi Columb. *2601.*

Additional collections and data allow the above expansion of the original description. This Aecidium, at present known only from the

Niobrara river valley in Nebraska, is distinctive, possessing cylindrical aecia and large, thick walled aeciospores. The telial stage perhaps occurs upon some Monocotyledonous host; quite possibly upon a sedge or a grass. One possibility, judging by the codistribution of host and rust, appeared to be *Puccinia eminens* Kern on *Carex Backii* Boot. An unreported culture in this laboratory, was, however, unsuccessful, and no definite morphological characters of the two species serve to indicate a relation.

2. AECIDIUM CIRCAEAE Cesati & Mont., in Montague, Syll. Gen. Spec. Crypt.: 312. 1856.

SYNONYMY: *Caeoma epilobiatum* Link, in Willd., Sp. Pl. 6^2: 59. 1825. (In part) *Aecidium Circaeae* Cesati in Rabenh. Herb. Mycol. No. 372. 1861.

LITERATURE: Winter, in Rabenh. Krypt. Fl. **1**: 266. 1881. Saccardo, Syll. Fung. **7**: 791. 1888. Schroeter, Pilze Schles. **1**: 379. 1889. Klebahn, Krypt. Mark Brand $\mathbf{5}^a$: 870. 1914.

This form, known only from Europe, on Circaea, has not yet been connected with a telial stage. Klebahn points to the fact that *Brachypodium silvaticum* Roem. & Schult. often grows in association, but he was unable to prove a connection with the rust *Puccinia Baryi* (Berk. & Br.) Winter. A comparison of these two forms fails to give a clue to a relationship between them.

The name *Caeoma epilobiatum* Link is used by Saccardo and by Klebahn as in part a synonym. This name is discussed further in this paper under *Puccinia Epilobii-tetragoni.*

3. PUCCINIASTRUM PUSTULATUM (Pers.) Dietel, in Engler & Prantl, Pflanzenfam. $\mathbf{1}^{1++}$: 47. 1897.

DESCRIPTION: N. Amer. Fl. **7**: 107. 1907.

LITERATURE: Saccardo, Syll. Fung. **7**: 762. 1888. Schroeter, Pilze Schles. **1**: 364. 1889. Klebahn, Krypt. Mark Brand. $\mathbf{5}^a$: 831. 1914. Sydow, Monogr. Ured. **3**: 444. 1915.

Pucciniastrum Abieti-chamaenerii Kleb. is united, in the North American Flora, with *Pucciniastrum pustulatum.* There are but slight differences in the morphological characters of the two species. Cultures, made in America since the publication of the description in the Flora, and substantiating European cultures, have been successful, however, only with the *Pucciniastrum Abieti-chamaenerii* form. It would seem, therefore, that the two forms might well now be considered as separate races.

Some confusion has existed concerning the synonymy of this species. Further study has shown that the synonymy, as given with the description in the North American Flora, should be revised as follows: *Uredo Epilobii* DC. in Lam. & DC. Fl. Franç. **6**: 73. 1815; and *Caeoma Epilobii* Link, in Willd. Sp. Pl. **6**^{2}: 29. 1825, are synonyms of *Puccinia Epilobii-tetragoni*, and are discussed in this paper under that species. In the place of *Caeoma Epilobii* in the Flora, should be listed *Caeoma Onagrarum* Link, in Willd. Sp. Pl. **6**^{2}: 29. 1825. (In part.) Following the latter name should be added *Erysibe pustulata Epilobium* Wallr., Fl. Crypt. Germ. **2**: 198. 1833. *Melampsora Chamaenerii* Rost., Medd. Bot. For. Kjöbenhavn. **1**: 77. 1884 (nomen nudum; no description) might be added to the synonymy, and also *Pucciniastrum Chamaenerii* Rostr., Plantepatol. 304. 1902.

The aecia of *Pucciniastrum pustulatum* (or, more accurately, of *Pucciniastrum Abieti-chamaenerii*) were unknown in America at the date of publication in the North American Flora. Fraser (Mycol. **4**: 176–177. 1912) first cultured this rust in America. Aecia have been found upon Pinaceae in America, and are represented in the herbarium as follows: on *Abies balsamea* (L.) Mill., Nova Scotia, Fraser, 1911; Michigan, Kauffman, 1914; Vermont, Orton, 1913; Wisconsin, Cheney, 1906; on *Abies concolor* (Gord.) Parry, Colorado, Bethel, 1903, 1909, 1913; on *Abies grandis* Lindl., Oregon, Jackson, 1915; Idaho, Weir, 1915; on *Abies lasiocarpa* (Hook.) Nutt., British Columbia, Holway, 1907; Oregon, Jackson, 1914; Colorado, Bethel, 1915.

The aecia of this species as found in America, produce spores somewhat larger than those of European collections, being 13–18 by 17–23 μ here, and but 10–14 by 13–21 μ in Europe. No other difference is noted.

The range of the uredinia and telia of this species has also been extended, the following additions having been made since the publication in 1907: to *Chamaenerion angustifolium* (L.) Scop. add New Mexico, Oregon, West Virginia; Alberta, British Columbia, Nova Scotia. To *Epilobium adenocaulon* Haussk. add Idaho, North Dakota, Oregon, South Dakota, Utah, Virginia, Washington, West Virginia, Wisconsin, Wyoming; British Columbia. Add *Epilobium affine* Bong., Alaska. To *Epilobium anagallidifolium* Lam., add Utah. Add *Epilobium novomexicanum* Hausskn., New Mexico; *Epilobium californicum* Hausskn., California; and *Epilobium brevistylum* Barbey, Oregon.

4. Pucciniastrum Circaeae (Thüm.) Speg., Dec. Mycol. Ital. No. 65. 1879.

Literature: Saccardo, Syll. Fung. 7: 763. 1888. Schroeter, Pilze Schles. 1: 364. 1889. Klebahn, Krypt. Mark Brand. 5^a: 833. 1914. Sydow, Monogr. Ured. 3: 445. 1915.

This European species upon Circaea presents no very tangible morphological differences from *Pucciniastrum pustulatum*. The hosts are different. Cultures, moreover, have so far failed to produce infection upon or from Abies, so that this species may well be considered to be distinct.

Klebahn (*l. c.*) gives some evidence for his suggestion that perhaps an overwintering of the rust occurs in the rhizomes of the host.

The synonym *Erysibe pustulata Circaeae* Wallr., Fl. Crypt. Germ. 2: 198. 1833, does not seem to appear in recent literature.

5. Uromyces plumbarius Peck, Bot. Gaz. 4: 127. 1879.

Descriptions: N. Amer. Fl. 7: 262. 1912. Sydow, Monogr. Ured. 1: 54–56. 1909.

This American species, treated here as in the North American Flora, represents a combination of four species described by different authors upon different species of host. This somewhat variable species falls into morphological races, corresponding in a general way with previously described species, as follows: (1) on *Gaura* (*Uromyces Gaurinus*). This race possesses teliospores of moderate size (16–23 by 24–33 μ), with the apex thickened the least of those of any of the races, 4–7 μ. A suggestion of verrucose markings can sometimes be seen upon the teliospores of this race. (2) On *Kneiffia* and *Oenothera* (*Uromyces Oenotherae*). This race is distinguished by dark-colored teliospores, with the apex the thickest of those of any of the races, 7–14 μ. The apex is often pointed, and the pedicel length the greatest in this species. (3) On *Luxauvia*, *Pachylophus*, and *Onagra* (*Uromyces plumbarius*). This race has the smallest teliospores (14–20 by 21–28 μ), with the apex but moderately thickened. The teliospores have been found to be very finely and inconspicuously verrucose, hardly noticeable unless the spores are viewed with the oil immersion. (4) On *Megapterium Fremontii* (*Uromyces Fremontii*). This race possesses teliospores with the thickest walls, sometimes to 3 μ. The teliospores are comparatively narrow and long.

The correlation of this species and these races with the corresponding autoecious long-cycled *Puccinia* is noted under *Puccinia Epilobii-tetragoni.*

The following additions may now be made to the range and hosts listed under the description in the Flora: add *Gaura induta* Woot. & Standley, New Mexico; add *Oenothera runcinata* (Engelm.) Small, New Jersey; to *Onagra biennis* add Delaware and Missouri; to *Pachylophus macroglottis* add Colorado; to *Pachylophus montanus* add Montana and Utah. To the exsiccati the following additions have appeared: Barth. Fungi Columb. *3893* and Barth. N. Am. Ured. *493*, *596*, *1396* and *1495*.

6. Puccinia Epilobii-tetragoni (DC.) Winter, in Rabenh. Krypt. Fl. **1**: 214. 1881.

Literature: Plowright, Monogr. Brit. Ured. 152–153. 1889. Sydow, Monogr. Ured. **1**: 423–435. 1903. Fischer, Beitr. Krypt. Schweiz 2^2: 152–153. 1904. McAlpine, Rusts Austral. 170. 1906. Holway, N. Amer. Ured. **1**: 74–79. 1907. Bubak, Pilze Boehmens **1**: 67. 1908. Grove, Brit. Rust Fungi 198–200. 1913. Lind, Danish Fungi 319. 1913. Klebahn, Krypt. Mark Brand. 5^a: 335–337. 1914. Saccardo, Syll. Fung., various volumes and pages, under the different names.

Synonymy:

Uredo vagans α Epilobii-tetragoni DC. Fl. Franç., **2**: 228. 1805.
Aecidium Epilobii DC. Fl. Franç., **2**: 238. 1805.
Uredo Epilobii DC. Fl. Franç., **6**: 73. 1815.
Puccinia pulverulenta Grev. Fl. Edinb. 432. 1824.
Caeoma Epilobii Link, in Willd. Sp. Pl. 6^2: 29. 1825.
Caeoma Epilobiatum Link, in Willd. Sp. Pl. 6^2: 59. 1825. p.p.
Puccinia tenuistipes Opiz, Seznam Rost. Kvét. Césté. 139. 1852.
Trichobasis Epilobii Berk. Outl. 333. 1860.
Puccinia Gayophyti Billings, in King Geol. Expl. 40th Par. **5**: 414. 1871.
Aecidium pallidum Schneid. Jahresb. Schles. Ges. 71. 1872.
Puccinia Oenthoerae Vize, Grev. **5**: 109. 1877.
Aecidium Gayophyti Vize, Grev. **7**: 12. 1878.
Puccinia Boisduvaliae Peck, Bot. Gaz. **7**: 45. 1882.
Puccinia Gayophyti Peck, Bot. Gaz. **7**: 56. 1882.
Puccinia Clarkiae Peck, Bull. Torrey Club **11**: 49. 1884.
Puccinia Epilobii Schroet. Pilz. Schles. **1**: 319. 1889.
Puccinia intermedia Diet. & Holw. Bot. Gaz. **18**: 254. 1893.
Puccinia heterantha Ell. & Ev. Erythea **1**: 204. 1893.
Puccinia Eulobi Diet. & Holw. Erythea **1**: 249. 1893.
Aecidium Clarkiae Diet. & Holw. Erythea **2**: 129. 1894.
Puccinia Sphaerostigmatis Diet. & Neg. Bot. Jahrb. Engler **22**: 353. 1896.
Dicaeoma Boisduvaliae Kuntze, Rev. Gen. Pl. 3^3: 468. 1898.
Dicaeoma Clarkiae Kuntze, Rev. Gen. Pl. 3^3: 468. 1898.

Dicaeoma Gayophyti Kuntze, Rev. Gen. Pl. 3^3: 468. 1898.
Dicaeoma heteranthum Kuntze, Rev. Gen. Pl. 3^3: 469. 1898.
Dicaeoma intermedium Kuntze, Rev. Gen. Pl. 3^3: 469. 1898.
Dicaeoma Oenotherae Kuntze, Rev. Gen. Pl. 3^3: 469. 1898.
Puccinia Gayophyti Speg. Anal. Mus. Nac. B. Ayres III. 1: 63. 1902.
Puccinia Zauchneriae Sydow, Monogr. Ured. 1: 435. 1903.
Puccinia glabella Holway, N. Amer. Ured. 1: 76. 1907.

O. Pycnia amphigenous, among or opposite the aecia, scattered, inconspicuous, subepidermal, honey-yellow becoming brown, globose, 85–150 μ in diameter by 110–170 μ in height; ostiolar filaments 30–65 μ long.

I. Aecia amphigenous, chiefly hypophyllous, scattered, from a diffused mycelium, numerous, often covering the entire leaf surface, cupulate or sometimes short-cylindrical, 0.2–0.5 mm. across; peridium white, margin recurved, lacerate; peridial cells rhomboidal, 13–22 by 22–36 μ, overlapping, the outer wall 5–10 μ thick, striate, the inner wall thinner, 3–6 μ, moderately verrucose; aeciospores irregularly globoid, angular, or ellipsoid, 13–20 by 13–23 μ (usually only to 20 μ in length); wall colorless, thin, 1 μ, minutely verrucose.

II. Uredinia amphigenous, often only hypophyllous, numerous, scattered, occasionally confluent, roundish, small, 0.1–0.8 mm. across, rather early naked, pulverulent, cinnamon-brown, ruptured epidermis noticeable; urediniospores ellipsoid. obovoid, or globoid, flattened slightly on two opposite sides, 15–26 by 19–31 μ; wall cinnamon-brown, thickness somewhat variable, 1.5–3 μ, moderately or sometimes closely echinulate, the pores 2, equatorial, rarely slightly superequatorial, in lighter colored areas in the flattened sides.

III. Telia amphigenous, sometimes caulicolous, numerous, scattered or sometimes confluent, roundish, rather small, 0.2–1 mm. across, early naked, pulverulent or sometimes compact, dark chestnut-brown, ruptured epidermis inconspicuous; teliospores ellipsoid or obovoid, rounded or somewhat narrowed at one or both ends, somewhat variable upon different hosts, 14–27 by 23–50 μ, usually somewhat constricted at the septum; wall cinnamon- or chestnut-brown, 1.5–3 μ thick, occasionally up to 4 μ thick, apex thicker, 4–12 μ, sometimes finely and inconspicuously verrucose; pedicel pale, rather fragile, usually broken away, but sometimes twice the length of the spore.

On Onagraceae:

Boisduvalia densiflora (Lindl.) Wats., California, Idaho, Oregon, Washington.
Boisduvalia densiflora imbricata Greene, California.
Boisduvalia glabella (Nutt.) Walp., Idaho, Nevada, Oregon.
Boisduvalia sparsiflora Heller, California.
Boisduvalia stricta (A. Gray) Greene (*B. Torreyi* Wats.), Oregon.
Chamaenerion latifolium (L.) Sweet (*Epilobium latifolium* L.), Alaska.
Chylisma cardiophylla (Torr.) Small (*Oenothera cardiophylla* Torr.), California.
Chylisma hirta A. Nels., Nevada.
Chylisma scapoidea scorsa A. Nels., Idaho.
Clarkia pulchella Pursh, Idaho, Oregon, Washington.
Epilobium adenocaulon Hausskn., Montana, New Mexico, Washington; Alaskal
Epilobium affine Bong., Alaska.

Epilobium clavatum Trel. Montana.

Epilobium minutum Lindl., Oregon.

Epilobium paniculatum Nutt., California, Colorado, Idaho, Montana, Nevada, North Dakota, Oregon, South Dakota, Utah, Washington, Wyoming.

Epilobium perplexans Trel., Idaho.

Eulobus californicus Nutt., California.

Gayophytum caesium Torr. & Gray, Idaho, Nevada, Utah, Wyoming.

Gayophytum diffusum Torr. & Gray, California, Idaho, Utah.

Gayophytum lasiospermum Greene, Utah.

Gayophytum Nuttallii Torr. & Gray, Idaho.

Gayophytum racemosum Torr. & Gray, Colorado, Idaho.

Gayophytum ramosissimum Torr. & Gray (*G. intermedium* Rydb.), Colorado, Idaho, Montana, New Mexico, Oregon, Utah, Wyoming.

Gayophytum sp., Arizona, Washington.

Godetia amoena (Lehm.) Lilja, California.

Godetia epilobioides (Nutt.) Wats., Nevada, Washington.

Godetia grandiflora Lindl., California.

Phaeostoma elegans (Dougl.) A. Nels. (*Clarkia elegans* Dougl.), California.

Phaeostoma rhomboidea (Dougl.) A. Nels. (*Clarkia rhomboidea* Dougl.), California, Washington.

Sphaerostigma andinum (Nutt.) Walp. (*Oenothera andina* Nutt.), Idaho, Washington.

Sphaerostigma bistorta (Nutt.) Walp. (*Oenothera bistorta* Nutt.), California.

Sphaerostigma Boothii (Dougl.) Walp. (*Oenothera Boothii* Dougl.), Oregon, Washington.

Sphaerostigma contortum (Dougl.) Walp. (*Oenothera contorta* Dougl.), California, Washington.

Sphaerostigma decorticans (H. & A.) Small (*Oenothera gauraefolia* Torr. & Gray), California.

Sphaerostigma dentatum (Cav.) Walp. (*Oenothera dentata* Cav.), Oregon.

Sphaerostigma hirtellum (Greene) Small (*Oenothera hirtella* Greene), California.

Sphaerostigma implexa A. Nels., Idaho.

Sphaerostigma micranthum (Hornem.) Walp. (*Oenothera micrantha* Hornem.), California.

Sphaerostigma pubens (S. Wats.) Rydb. (*Oenothera strigulosa pubens* S. Wats.), California.

Sphaerostigma spirale (Lehm.) Walp. (*Oenothera spiralis* Hook.). California.

Sphaerostigma utahense Small, Utah.

Sphaerostigma Veitchianum (Hook.) Small (*Oenothera bistorta Veitchiana* Hook.), California.

Sphaerostigma viridescens (Lehm.) Walp. (*Oenothera viridescens* Lehm.), California.

Taraxia brevifolia Nutt., Montana.

Taraxia graciliflora (H. & A.) Raim. (*Oenothera graciliflora* H. &. A.), California.

Taraxia heterantha (Nutt.) Small, Wyoming.

Taraxia longiflora Nutt. (*Oenothera Nuttallii* Torr. & Gray), Nevada.

Taraxia ovata (Nutt.) Small (*Oenothera ovata* Nutt.), California.

Taraxia subacaulis (Pursh) Rydb. (*Oenothera heterantha* Nutt.), Colorado, Idaho, Montana, Nevada, Utah, Wyoming.

Zauchneria californica Presl., California.

Zauchneria Garrettii A. Nels., Utah.

Type Locality: France, on *Epilobium tetragonum.*

Distribution: From the western part of the Dakotas westward to the coast, and from Alaska to New Mexico and California; also (in part) in Europe, Asia, Australia, and South America.

Illustrations: Holway, N. Amer. Ured. 1: *pl. 33, f. 113a & b; pl. 34, f. 113c to e, 114, 115a & b; pl. 35, f. 115 c to h; pl. 36, f. 116 and 117.* Beitr. Krypt. Schweiz 2²: *f. 118.*

Exsiccati: Barth. Fungi Columb. *2469, 2558, 2771, 3750, 3752, 4767*; Barth. N. Amer. Ured. *159, 295, 341, 356, 438, 439, 856, 953, 1148, 1252, 1262, 1350, 1359, 1440, 1579*; Clements, Crypt. Form. Colo. *561*; Ell. & Ev. Fungi Columb. *1851*; Ell. & Ev. N. Amer. Fungi *1846, 2986, 2995, 3139, 3140, 3477,* Garrett, Fungi Utah. *49, 50, 86, 92, 110, 145, 162, 173*; Sydow, Ured. *864, 865, 866, 874, 875, 881, 1063, 1064, 1768, 1918, 1919.*

As the synonymy indicates, some fifteen separately described and named long-cycled autoecious species of *Puccinia* upon the Onagraceae have been here combined as one species. Several of these names have been considered to be synonyms by different authors. For example, Holway considers *Puccinia pulverulenta* and *Puccinia intermedia* to be synonymous with *Puccinia Epilobii-tetragoni*, and *Puccinia Boisduvaliae*, *Puccinia Clarkiae*, *Puccinia Eulobi* and *Puccinia Sphaerostigmatis* to be hardly distinguishable from *Puccinia Oenotherae. Puccinia Gayophyti* has been described three separate times by independent authors. Other earlier repetitions in description, under the same names, are not included.

It was not without some hesitation that it was decided thus to combine all these forms. In first going over a few specimens of each species, for the sake of comparison, it was obvious that considerable differences existed. Some of the specimens showed dark, chestnut-brown teliospores, others lighter, cinnamon-brown; some specimens showed teliospores with scarcely thickened apices, others, thickened to 12 or 13 μ; some were verrucose, others smooth; the size of these spores varied considerably. It seemed that surely several species of rust existed upon these hosts, as other workers had concluded; to consider combining them appealed to the writer as an easy and therefore inexcusable dodging of the issue. It was remembered, further, that this rust upon Epilobium occurs over most of the world; upon most of the other hosts, only in America. However, as the study progressed, other considerations were forced upon the attention of the writer: as already noted, one has here to deal with closely related, variable hosts, and it is perhaps not strange that one should find the rusts also to be variable, and that the related American hosts should bear related rusts. In the course of the study here, between two and three hundred specimens of these autoecious rusts in the Arthur herbarium, assigned tentatively to different specific names, were examined. With the continuation of the work, it became more and more evident that the differences separating a few collections were neither of sufficient value nor constancy, as evidenced by an examination of a large number of collections, to render possible a division of species upon a morphological basis. A few examples may aid in corroborating this view.

The host Boisduvalia affords the most striking evidence of the variability of the rusts upon the Onagraceae. Holway, in his work upon the North American Uredineae, very logically described as a new species, *Puccinia glabella*, the rust occurring upon *Boisduvalia glabella* (Nutt.) Walp. This description was from a specimen distributed by Griffiths, West American Fungi 385, as *Puccinia Boisduvaliae* Peck. Holway points out that this rust upon *Boisduvalia glabella* is a very different thing from the rust occurring upon other species of *Boisduvalia: Puccinia glabella* has small teliospores (Holway gives 15–18 by 25–32 μ), with the apex not at all or only slightly thickened; *Puccinia Boisduvaliae* possesses larger teliospores, usually much thickened at the apex. In a recent visit to the University of Wyoming, however. Dr. Arthur obtained further specimens of rusts upon Onagraceous hosts. Among these was a collection upon *Boisduvalia glabella* showing somewhat larger teliospores, with thicker apices, than was shown by other specimens in the herbarium upon this host. The measurements of the teliospores of this specimen of *Puccinia glabella* were 15–19 by 26–35 μ, the apex 4–7 μ. From the same herbarium a specimen was secured upon *Boisduvalia densiflora* (Lindl.) Wats., with spores but little larger, 16–23 by 32–39 μ, and the apex the same thickness, 4–7 μ. Another collection in the herbarium upon *Boisduvalia densiflora* shows teliospores 18–26 by 32–53 μ, with the apex 7–12 μ. That an error in identification of the host is not the explanation here, is indicated, not only by an examination of the material, and a consideration of the carefulness of the collectors, but by the fact that other specimens of Boisduvalia rusts show similar variability; in fact, a gradual gradation is to be noted. Here then, the two extremes in these forms of Onagraceous rusts occur upon the same genus of host, to a great extent upon the same species, and shade by degrees the one into the other.

The genus Chylisma shows much variation in the rust upon different species; for example, a collection upon *Chylisma hirta* A. Nels. possesses teliospores 21–24 by 37–50 μ, the wall chestnut-brown, smooth, the apex 7–11 μ, the pedicel once or twice the length of the spore; another collection upon *Chylisma scapoidea scorsa* A. Nels. shows teliospores but 16–20 by 26–34 μ, the wall from cinnamon- to chestnut-brown, appearing almost as if verrucose, the apex but 4–7 μ thick, the pedicel short.

The evidence obtained from the large number of collections points unquestionably toward great variability in morphological characters within the forms upon these Onagraceous hosts. In a general way differences are found between the rusts upon these different hosts which ordinarily may be utilized to indicate the placing of the rust within morphological races, as is indicated later in this article; yet specific distinctions, it seems to the writer, can not be drawn. It is significant that, in the work upon the correlated long-cycled autoecious Uromyces forms upon the Onagraceae, of which four species had been described, it was considered desirable also to combine them, as already published in the North American Flora.

The long synonymy involves some names about which confusion exists in the literature. The name *Aecidium pulchellum* Schrad. is omitted from the synonymy, being listed by DeCandolle as a synonym of his *Aecidium Epilobii* in Fl. Fr. **2**: 238. 1805. As no reference to any publication is given, it may be assumed that *Aecidium pulchellum* was a manuscript or herbarium name, never established. *Puccinia tenuistipes* Opiz is included on the authority of Sydow, who states that it occurs on *Epilobium hirtum* and is fully identical with *Puccinia Epilobii-tetragoni*. As listed by Opiz, Seznam Rost. Kvet. Ceske 139. 1852, one can hardly determine what is referred to. *Aecidium pallidum* Schneid. is also included on authority of Sydow (*l. c.*) also Schroeter (*l. c.*, p. 319), it having been found that Schneider erroneously determined his host plant, considering it to be *Lythrum Salicaria*, whereas Schroeter finds the host in reality to be *Epilobium hirsutum*.

Puccinia Epilobii DC. Fl. Fr. **6**: 61. 1815, is used by Saccardo, Syll. Fung. **7**: 608. 1888, and by others, as the name for this species here considered as *Puccinia Epilobii-tetragoni*. The original *Puccinia Epilobii* of DeCandolle refers, however, to the short-cycled species still known under that name. Saccardo in Syll. Fung. **17**: 245. 1905, corrects the earlier mistake. Kuntze has made the combination *Dicaeoma Epilobii* Rev. Gen. **3**^{3}: 469. 1898, which name may be considered to be a synonym of the short-cycled *Puccinia Epilobii* DC.

The names *Uredo Epilobii* DC. and *Caeoma Epilobii* Link, included in the synonymy, have been particularly confused in the literature. Both these names have been used in the North American Flora as synonyms of *Pucciniastrum pustulatum* (Pers.) Dietel, and both have been used by the Sydows (Monog. Ured.) as synonyms both of *Pucciniastrum pustulatum* and *Puccinia Epilobii-tetragoni*. *Uredo Epilobii* is described by DeCandolle to take the place of his *Uredo vagans* var. *Epilobii-tetragoni*, which latter name he includes as a synonym. DeCandolle further says in his description that this Uredo is often found associated with *Aecidium Epilobii*, and he contrasts *Uredo Epilobii* with *Uredo pustulata* and *Puccinia Epilobii*. It is evident that this name in question refers to *Puccinia Epilobii-tetragoni* and not to a Pucciniastrum. *Caeoma Epilobii* is given by Link as having for synonyms *Uredo Epilobii* DC. and *Uredo vagans Epilobii* DC. (meaning *Uredo vagans Epilobii-tetragoni*, since he gives as reference Fl. Fr. **2**: 228). Link briefly describes *Caeoma Epilobii* as possessing ruptured epidermis surrounding, and as occurring upon *Epilobium tetragonum*. This name very evidently must be considered to be a synonym of *Puccinia Epilobii-tetragoni*.

Caeoma Epilobiatum is given by Link with *Aecidium Epilobii* DC. as a synonym. This Caeoma name has been used by the Sydows and others as a synonym of *Puccinia Epilobii-tetragoni*. It has also been used as a synonym of *Aecidium Circaeae* Cesati and Mont., as indicated previously in this paper. Link describes his species as "maculis oblitteratis," with a pseudoperidium, and orange or yellow spores, and as inhabiting leaves of Epilobium and Circaea in Europe. It would seem that, as Klebahn and others indicate, this name of Link may be used as a synonym of *Aecidium Circaeae* in part, and that it might refer also in part to *Puccinia Epilobii-tetragoni*.

The majority of the synonyms refer to descriptions made when this rust, with apparent differences, was found upon a new host, and to combinations made from these names. It is only when a large number of collections are at hand that the value of the differences, indicated by the various descriptions, can be determined.

Some reasons for the continuation of the specific name *Epilobii-tetragoni* may not be out of place here. While used first as a varietal name, and resulting now in a long trinomial, its retention seems advisable in view of common usage and establishment by leading authorities. Furthermore, the two names following this in priority can scarcely be used, the Puccinia combination referring to a short-cycled form. Plowright and Grove have used the name *Puccinia pulverulenta* Grev., but this latter name is hardly in use outside of Great Britain.

Pycnia are not frequent in this species, since, as has been often pointed out, the aecial mycelium is perennial. Pycnia are found, it may be supposed, when infection with basidiospores occurs. Plowright (Monog. Ured. 152. 1889) states that in 1882 he obtained aecia from the sowing of aeciospores upon *Epilobium hirsutum.* Grove, however, doubts the validity of the result. Indeed, it is hardly to be expected that a rust should have two repeating spore stages; yet probably not beyond the bounds of possibility. Dietel (Flora **81**: 401. 1895) discusses this production of secondary aecia, and states that he obtained uredinia from sowing aeciospores. The aecia vary somewhat in shape, depending upon conditions, being sometimes short cylindric, usually cupulate, sometimes oval. The aeciospores vary slightly in size. It has been found rather infrequently that the aeciospores attain a length of 23 μ, ordinarily being only up to 20 μ in length. This is especially true in cases in which uredinia or telia are present with the aecia. As far as can be determined from collections and studies thus far made, no definite distinction can be found between the aecia of *Puccinia Epilobii-tetragoni* and the aecia of *Puccinia Veratri,* save that the aeciospores of collections placed with the latter are ordinarily slightly larger, being more frequently up to 23 μ in length. These points are touched upon under *Puccinia Veratri* in this article.

It is, of course, possible that mixed infections may occur upon species of Epilobium. The aecia of *Puccinia Veratri* and the uredinia and telia of *Puccinia Epilobii-tetragoni* might easily be found occurring upon the same plant. Field collections of such infections would ordinarily be considered as only *Puccinia Epilobii-tetragoni.*

The uredinia and urediniospores are rather constant for this species. Such variations as occur are considered in the discussion of the races.

The telia and teliospores show the greatest variation. It has been chiefly upon teliospore characters that specific differences have been previously indicated. Some telia are pulverulent, some more compact. There is considerable variation as to size of telia. The teliospores, in some collections and upon some species of host, are somewhat verrucose; the apical thickening, the size, the pedicel length, and in a measure, the color, likewise vary. While these variations are not altogether constant, some of them can be made use of in separating races, as is indicated later.

Holway (N. Amer. Ured. **1**: 76) points out that *Puccinia Epilobii-tetragoni* shows a tendency to produce only aecia and telia at higher altitudes. This is a condition that has been previously observed with rusts. (See Magnus[8] and Fischer,[9] and further data under *Puccinia Epilobii-Fleischeri.*)

Intermingling of the perennial mycelium producing aecia and the local mycelium producing uredinia and telia, is frequently evidenced. So too, cases are found in which local gametophytic and sporophytic mycelia are intermingled, pycnia, aecia, and uredinia or telia or both, being sometimes found close together upon the same leaf. This point affords an opportunity for further study.

South American, European, and other foreign collections of this rust, upon Epilobium, as represented in the Arthur herbarium, show no morphological characters different from those of North American collections.

From the data at hand, as previously indicated, it appears that *Puccinia Epilobii-tetragoni* is a very variable species; these variations can be made use of in assigning morphological races to this species. To Mr. C. A. Ludwig thanks are due for a certain amount of the preliminary work pertaining to the separation of these races. The races are, in general, as follows:

1. *Puccinia Gayophyti* race. This race occurs upon Gayophytum (*Puccinia Gayophyti*) and Chamaenerion and Epilobium (*P. Epilobii-tetragoni, P. pulverulenta, P. intermedia*). The hosts of this race are very similar as to vegetative characters; so too are the forms of this

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[8] Magnus, P., Bericht. Deutsch. Bot. Ges. **11**: 453–464. 1893, and Hedw. Beibl. **39**: 147–150. 1900.

[9] Fischer, Ed., Verh. Schweiz Nat. Ges. Luzern **88**: 47. 1906.

rust which they bear. This race is quite uniform and unvariable, and almost of world wide distribution. The apex of the teliospore is, in this race, but moderately thickened, 3–7 μ, the wall cinnamon-brown, often finely verucose. Holway separates *Puccinia Epilobii-tetragoni* upon Epilobium from *P. Gayophyti* chiefly by the position of the germ pores of the teliospores, which in *Puccinia Epilobii-tetragoni*, as he considered the species, he finds are one or two in the lower cell, midway between the septum and base of the spore. This distinction in general holds, but has not been found of absolute constancy. This race is rather well correlated with the *Uromyces Gaurinus* race of *Uromyces plumbarius*.

2. *Puccinia glabella* race, on *Boisduvalia glabella*. But few specimens have been collected from this host. This race possesses the the smallest teliospores, 22–39 μ in length, with the apex but slightly thickened, 2–7 μ, the wall cinnamon-brown, rather paler below. The urediniospores have rather thick walls, 1.5–3 μ. This seems on the whole to be a good race, although many collections of the *Puccinia Gayophyti* race show characters quite as in this race, and other Boisduvalia rusts sometimes approach this.

3. *Puccinia heterantha* race, on Taraxia. This race is distinguished by thick walled teliospores, 1.5–3 μ, rather dark in color, cinnamon- to chestnut-brown, with the apex but moderately thickened, 3–7 μ. Considerable evidence of correlation between the *Uromyces Fremontii* race of *Uromyces plumbarius* is shown.

4. *Puccinia Oenotherae* race. On Boisduvalia [excepting *Boisduvalia glabella*] (*Puccinia Boisduvaliae*), Clarkia, Phaestoma (*Puccinia Clarkiae*), Chylisma, Godetia, Oenothera (*Puccinia Oenotherae*), Eulobus (*Puccinia Eulobi*), Sphaerostigma (*Puccinia Sphaerostigmatis*) and Zauchneria (*Puccinia Zauchneriae*). This group of hosts possesses rusts with thicker walled and darker teliospores, as in the preceding race, but with the apical thickening often greater, 4–12 μ, the thickening dark colored. The pedicel is sometimes up to 100 μ in length, the urediniospore walls thick. This race is correlated in particular with the *Uromyces Oenotherae* race of *Uromyces plumbarius*, although the *Uromyces plumbarius* and the *Uromyces Fremontii* races are not greatly different.

The above races of the species *Puccinia Epilobii-tetragoni* are not in all cases exactly correlated with the separate *Uromyces plumbarius* races, since no corresponding parallel of hosts, distribution, etc., fully exists. The *Puccinia Oenothera* race differs from the *Uromyces Oenotherae* race only in the possession of two-celled teliospores. *Puccinia Epilobii-tetragoni* as a whole may well be said to be correlated with *Uromyces plumbarius* as a whole, as well as with *Puccinia Epilobii-Fleischeri.* The aecia in all three species are very similar to those of *Puccinia Veratri.* Possible correlations with short-cycled species of Puccinia are noted further with the discussion of such forms.

These races are separated out on morphological grounds, chiefly. Klebahn states (*l. c.*) that the biological identity of the forms upon different species of Epilobium, awaits proof; certainly the biological status of the collective species as here described, awaits study. The ultimate biological or physiological races may or may not follow these indicated morphological races; but it seems convenient at this time tentatively to designate such races.

Puccinia Krookii P. Henn., Ann. Naturhist. Hofmus. Wien for 1900: 1, described as on Epilobium sp., Harrysmith, Natal, and *Puccinia luxurians* Dietel & Neger, Engler's Jahrb. 24: 158. 1900, on *Oenothera mutica*, Cordillera de Santiago, Chile, present, in the descriptions, no distinctions that can be made use of in separating them from *Puccinia Epilobii-tetragoni* as here described. Both are listed in Sydow, Monog. Ured. Since no specimens are at hand for comparisons, however, their status cannot definitely be decided.

7. PUCCINIA VERATRI Duby Bot. Gall. **2**: 890. 1830.

LITERATURE: Holway, N. Amer. Ured. **1**: 21. 1905. Sydow, Monogr. Ured. **1**: 639. 1903. Winter, in Rabenh. Krypt. Fl. **1**: 184. 1881. Saccardo, Syll. Fung. **7**: 665. 1888. Fischer, Beitr. Krypt. Schweiz 2^2: 81. 1904. Oudemanns, Ann. Mycol. **2**: 358. 1904. Tranzschel, Ann. Mycol. **7**: 182. 1909. Klebahn, Krypt. Mark Brand. 5^a: 338. 1914.

O. Pycnia hypophyllous, scattered between the aecia, immersed, becoming brownish, globose or flask shaped, rather large, 112–144 μ in diameter by 128–175 μ in height; ostiolar filaments 55–65 μ long; pycniospores many, oval, 0.5–1 by 1–3 μ.

I. Aecia hypophyllous, numerous, crowded often over the entire lower surface of the leaf, broad cupulate, 0.3–0.6 mm. in diameter; peridium white, much recurved, the margin lacerate; peridial cells rhomboidal or oblong, 16–21 by 21–30 μ, somewhat overlapping, the outer wall 3–5 μ thick, striate, the inner wall 3–6 μ thick, verrucose; aeciospores roundish or oval, 14–18 by 16–24 μ; wall light yellow, thin, 1 μ, finely verrucose.

ON ONAGRACEAE:

Chamaenerion latifolium (L.) Sweet (*Epilobium latifolium* L.), British Columbia.

Epilobium alpinum L., New Hampshire, Utah.
Epilobium Hornemanii Reich., Utah; British Columbia.
Epilobium paniculatum Nutt., Idaho; Washington.
Epilobium rubricaule Rydb., Utah.
II and III. Described in literature indicated.

Oudemanns (*l. c.*) has clarified the situation in regard to the author of this name, Niessel being often given credit for the name. So, too, Sydow's use of the name *Puccinia Veratri* Duby as a synonym for *Uromyces Veratri* (DC.) Schroet., is in error.

Tranzschel (*l. c.*) established the connection of the form with uredinia and telia on Veratrum with these aecia on Epilobium, obtaining his clue from the similarity of teliospores of this species with those of *Puccinia Epilobii*. In America it had for some time been noted that aecia occurred upon Epilobium without being followed by telia. These aecia agreed in general with the aecia of *Puccinia Epilobii-tetragoni*, however; they were therefore usually referred to that species. Actual cultures have not been reported for America showing the connection with *Puccinia Veratri* in such cases, but it seems logical to assume that such aecia are the alternate phase of this *Puccinia Veratri*. As noted in the discussion of *Puccinia Epilobii-tetragoni*, differences in aecia referred to these two species are small indeed. The aecia can scarcely be considered to be local. In truth, it has been more upon the fact that telia did not follow aecia, telia upon Veratrum being at hand, or sometimes because of proximity of collections of the two forms, that led to the aecial specimens being considered to be *Puccinia Veratri*.

Descriptions of the aecia for this heteroecious species have not been found in the literature.

There is a foreign Uromyces on Veratrum, with smooth teliospores and thickened apex, evidently not correlated with *Puccinia Veratri*.

8. PUCCINIA PECKII (DeToni) Kellerman, Journ. Mycol. **8**: 20. 1902.

O & I. Described as *Aecidium Oenotherea* by Peck in Rep. N. Y. State Mus. **23**: 60. 1873. (See Sacc. Syll. Fung. **7**: 790. 1888.)
II and III. On several species of Carex.

The aecia of this species have been found upon Gaura, Onagra, Merolix and Pachylophus from many parts of North America, especially from the central plains area. The aecia are distinguishable, especially from those of *Uromyces plumbarius*, which occur upon some of the same hosts, by their local character. The aecia are rather large, the aeciospores up to 21 μ in diameter, with thin walls.

This species is shortly to be discussed in some detail by Kern.

As is indicated later in this article, the teliospores of this species, upon Carices, resemble the teliospores of certain other rusts upon the Onagraceae.

9. PUCCINIA JUSSIAEAE Speg., Anal. Soc. Cienc. Argentina **12**: 68. 1881.

(*Puccinia Ludwigiae* (Ell. & Ev.) Holway, N. Amer. Ured. **1**: 72. 1907.)

LITERATURE: Ell. & Ev., Proc. Acad. Phila. 155. 1893. Ell. & Ev., Bull. Torrey Club **22**: 363. 1895. Saccardo, Syll. Fung. **14**: 298. 1899; **21**: 627. 1912. (See also below.) Sydow, Monogr. Ured. **1**: 438. 1903. Spegazzini, references below.

Ellis and Everhart in 1895 described this Puccinia as on *Nesaea verticillata*, which was an error for *Ludwigia polycarpa*. (See Holway, *l. c.*) The name *Puccinia Nesaeae* was used, and perpetuated by Saccardo and Sydow. Ellis and Everhart had previously (1893), however, described the aecial stage of this fungus as *Aecidium Ludwigiae*.

This rust occurs upon various species of Ludwigia (Isnardia) in the central and southeastern portions of the United States.

With this species, formerly known as *Puccinia Ludwigiae*, is placed *Aecidium Jussiaeae* Speg. and *Puccinia Jussiei* Speg., making the name for this species *Puccinia Jussiaeae*. C. R. Orton, in working upon *Puccinia Ludwigiae*, discovered that the above rusts upon Jussiaea agree exactly, so far as can at present be determined, with the Ludwigia rust. Furthermore, Spegazzini states that the Aecidium is found associated with the Puccinia stage upon Jussiaea, thus making the rust one with the same life cycle as *Puccinia Ludwigiae*. Also, according to Engler and Prantl, Jussiaea and Ludwigia are very closely related and similar plants. *Aecidium Jussiaeae* was described in Anal. Soc. Cienc. Argentina **9**: 174. 1880; also in Saccardo, Syll. Fung. **7**: 790. 1888, and distributed by Spegazzini as Dec. Myc. Argentinae *30*. This specimen is in the herbarium here. *Puccinia Jussiei* was described in Anal. Soc. Cienc. Argentina **12**: 68. 1881; then in Sacc. Syll. Fung. **7**: 700. 1888. The hosts given are *Jussiaea lanceolata* and *Jussiaea longifolia*. No specimens of this Puccinia are at hand, but the description agrees almost exactly with that of *Puccinia Ludwigiae*.

To Mr. Orton are also due thanks for finding that *Aecidium Isnardiae* Lagerh. Tromso Mus. Aarsh. **17**: 102. 1895, described as upon leaves of Isnardia from Ohio, collector uncertain, belongs here. Isnardia is a synonym of Ludwigia. Farlow, Bibl. Index $\mathbf{1}^1$: 59. 1905, gives some further data regarding *Aecidium Isnardiae.*

Puccinia Jussiaeae Speg., being the oldest name for this species, is, therefore, to be used.

This species, with long, narrow teliospores, does not show a correlation with *Puccinia Epilobii-tetragoni.* It is more definitely correlated with *Puccinia Circaeae,* as is discussed under the short-cycled species, in this paper.

10. PUCCINIA EPILOBII-FLEISCHERI Ed. Fischer, Bull. Herb. Boiss. 1897: 394. 1897.

LITERATURE: Saccardo, Syll. Fung. **14**: 299. 1899. Sydow, Monogr. Ured. **1**: 426. 1903. Fischer, Beitr. Krypt. Schweiz $\mathbf{2}^2$: 154–155. 1904.

This species, known only from Europe upon *Epilobium Fleischeri* Hochst. (*Chamaenerion Fleischeri* Fritsch.) is without a uredinial stage. As Fischer (*l. c.*) states, it is nearly related to *Puccinia Epilobii-tetragoni*, excepting in the lack of uredinia. Fischer notes some small differences in the telial stages of the two species; yet this is no doubt correlated with *Puccinia Epilobii-tetragoni*, as previously indicated.

It seems to be established that uredinia do not occur in *Puccinia Epilobii-Fleischeri;* Fischer lists several collections bearing aecia and telia together. It is worthy of note that this species occurs at high altitudes, *i. e.*, in Switzerland. As already noted, uredinia of *Puccinia Epilobii-tetragoni* often occur less abundantly at higher altitudes in western North America. While no host-species of *Epilobium* has been found there which conspicuously lacks in the development of the uredinial stage, and while the evidence of the fixity of such a character in America is lacking, it is to be expected that a form agreeing with *Puccinia Epilobii-Fleischeri* may be found in the higher western portions of our continent.

Cultures have, apparently, not yet been made to decide just what the life cycle is in *Puccinia Epilobii-Fleischeri* under various conditions.

The short-cycled species of Puccinia upon the members of the Onagraceae present some difficulties. This is more directly due to the fact that, of these forms, only *Puccinia Circaeae* has been collected in America in sufficient numbers to render its status definite. There is a considerable variation, in the morphological characters, between these different short-cycled forms. These characters appear to indicate evident relationships or correlations with different long-cycled species of rust upon the same or similar hosts. As is indicated under each species, and discussed further on in this article, these short-cycled forms appear to fall into two, very doubtfully three, general groups: the first, represented by *Puccinia Circaeae*, *Puccinia gigantea*, and *Puccinia Fuchsiae*, shows resemblance to *Puccinia Jussiaeae*. The second group, represented by *Puccinia Epilobii* and *Puccinia scandica*, shows a relationship to *Puccinia Veratri* and to *Puccinia Epilobii-tetragoni*. The third, represented by *Puccinia sphaeroidea*, is distinctive, but evidently does not in reality belong among the rusts of the Onagraceae.

11. PUCCINIA CIRCAEAE Pers. Tent. Disp. Fung. 39. 1797.

LITERATURE: Saccardo, Syll. Fung. 7: 686. 1888. Schroeter, Pilz. Schl. 1: 348. 1889. Sydow, Monogr. Ured. 1: 422. 1903. Fischer, Beitr. Krypt. Schweiz 2^2: 319. 1904. Holway, N. Amer. Ured. 1: 79. 1907. Klebahn, Krypt. Mark Brand. 5^a: 552. 1914.

This cosmopolitan species occurs upon all the species of Circaea present in North America, the rust probably being coextensive with the host. It frequently has been noted that the teliospores germinate both as a micro- and a lepto-Puccinia, depending upon the season.

The teliospores in this species are shorter and narrower than those of *Puccinia Jussiaeae*, yet it would seem that these are correlated species. The micro-form bears a greater resemblance to *Puccinia Jussiaeae* than does the lepto-form.

Schweinitz, Schr. Nat. Ges. Leipzig 1: 70. 1822, listed *Uredo Circaeae* as occurring in Carolina, then in Trans. Amer. Phil. Soc. n. ser. 4: 291. 1832, gave the name as *Caeoma Uredo Onagrarum* Link, and Pennsylvania also as a locality. The names he gives are now considered to refer to *Pucciniastrum Circaeae*, a species which does not occur, so far as is known, in North America. In both the above mentioned publications Schweinitz also lists *Puccinia Circaeae*. Further information is being sought from the Schweinitz herbarium in Philadelphia; until an examination of the original material is made, if such a thing be possible, it may not be unreasonable to assume that the somewhat different appearance of this rust in the micro- and leptoform, may have led to the supposition that an Uredo occurred upon Circaea.

12. PUCCINIA GIGANTEA Karst., Mycol. Fenn. **4**: 42. 1878.

LITERATURE: Saccardo, Syll. Fung. **7**: 669. 1888. Ellis & Everhart, Bull. Torrey Club **27**: 60. 1900. Sydow, Monogr. Ured. **1**: 428. 1903. Fischer, Beitr. Krypt. Schweiz 2^2: 320. 1904. Holway, N. Amer. Ured. **1**: 74. 1907. Klebahn, Krypt. Mark Brand. 5^a: 553 (note). 1914.

Holway places with this species *Puccinia annulata* Ell. & Ev., both of which occur upon *Chamaenerion angustifolium* (L.) Schur. (*Epilobium angustifolium* L.). *Puccinia annulata* was described as possessing smaller teliospores, yet the two species no doubt go together, *Puccinia annulata* being but an American variation of the European *Puccinia gigantea.*

This species is not greatly different from *Puccinia Circaeae,* and appears to be correlated with *Puccinia Jussiaeae.* Only a very few collections are at hand, however.

13. PUCCINIA FUCHSIAE Sydow & Holway; Sydow, Ann. Mycol. **4**: 30. 1906.

LITERATURE: Holway, N. Amer. Ured. **1**: 79. 1907. Saccardo, Syll. Fung. **21**: 627. 1912.

This species, as far as is known, has only been collected once, at Amecameca, Mexico. Sydow, *l. c.*, suggests that this species is much like *Puccinia Jussiaeae* from South America. It may, however, quite probably be a correlated short-cycled form. An examination of the original material discloses no very obvious difference from *Puccinia gigantea.* The host listed is *Fuchsia thymifolia.*

14. PUCCINIA EPILOBII DC., Fl. Fr. **6**: 61. 1815.

LITERATURE: Schroeter, Pilze Schles. **1**: 319. 1889. Sydow, Monogr. Ured. **1**: 427. 1903. Saccardo, Syll. Fung. **17**: 348. 1905. Holway, N. Amer. Ured. **1**: 73. 1907. Bubak, Archiv. Naturw. Land. Boehmen **13**: 148. 1908. Lind, Danish Fungi 318. 1913. Klebahn, Krypt. Mark Brand. 5^a: 337. 1914.

This species, upon several species of Epilobium, is rather common in Europe. In America only two specimens referred to this species are known to have been collected (Holway, *l. c.*). Lind points out that the mycelium is perennial in the subterranean portions of the

the hots; sori are therefore scattered. The teliospores are verrucose, the walls usually uniformly 2 μ thick. As previously indicated, Klebahn directed attention to the similarity of these teliospores to those of *Puccinia Veratri*. While this micro-form thus corresponds with the long-cycled *Puccinia Veratri*, the relation with *Puccinia Epilobii-tetragoni*, especially with some collections possessing less thickened apices, is evident. The correlations thus evidenced, belong, then, to both the second and third types as indicated early in this paper.

15. Puccinia scandica Johans., Bot. Centralbl. **28**: 395. 1886.

Literature: Saccardo, Syll. Fung. **7**: 680. 1888. Sydow, Monogr. Ured. **1**: 427. 1903. Holway, N. Amer. Ured. **1**: 73. 1907.

Specimens referred here have been collected a few times in North America. The chief difference from *Puccinia Epilobii* is in the smaller size of the teliospores, and the rather thicker apices, in collections considered to be *Puccinia scandica*. *Epilobium alpinum* and *Epilobium clavatum* are the hosts known in North America. *Epilobium alpinum* is also an American host for what is considered to be *Puccinia Epilobii*. While the differences between these two short-cycled rusts appear to hold for North America so far as the few collections are concerned, further collections may possibly indicate that these two species belong together. This species shows more definitely the correlation with *Puccinia Epilobii-tetragoni*, especially the race upon Epilobium, and may also be considered to be correlated with *Puccinia Veratri*.

16. Uredo oenothericola Speg., Anal. Mus. Nac. B. Aires **19**: 318. 1909.

Literature: Saccardo, Syll. Fung. **21**: 794. 1912. Spegazzini, Anal. Mus. Nac. B. Aires **23**: 32. 1912.

Spegazzini described this rust upon *Oenothera mollissima*, from South America. He refers again to it in his later publication cited. No specimens have been seen by the writer. No clues are evident from his description. What the significance is, of his statement "pedicello hyalino mox fatiscente (40–50 μ lng. 5 μ crss.) saepe suffultae," is a question. It is not impossible that this rust may be the uredinial stage of some previously noted rust upon Oenothera.

DOUBTFUL SPECIES

17. PUCCINIA SPHAEROIDEA P. Henn. Hedwigia **42**: (107). 1903.

LITERATURE: Sydow, Monogr. Ured. **1**: 890. 1904. Saccardo, Syll. Fung. **17**: 348. 1905. Holway, N. Amer. Ured. **1**: 72. 1907.

This species, cited as upon Jussiaea sp., Lower California, differs markedly from any other rust upon the Onagraceae. The collection by Purpus, 1902, is the only one known. The specimen represented in the herbarium here is very fragmentary. The teliospores are ovoid, the wall uniformly thick, the pedicel persistent, often inserted laterally. These teliospores exactly resemble those of *Puccinia sphaerospora* Sydow and Henn., the hosts of which are Asclepiadaceous plants. Sections of the material indicate that the host is quite likely in reality some Asclepiad. The characteristic spores point strikingly toward such a conclusion. On the whole, this species seems most doubtfully to belong with the Onagraceous rusts.

EXCLUDED SPECIES

PUCCINIA COLUMBENSIS Ell. & Ev. Proc. Acad. Phila. 1893: 153. 1893.

Stated to be upon *Oenothera biennis*. Holway, Journ. Mycol. **8**: 171. 1902, points out that the host of this Puccinia is Troximon, not Oenothera. An Aecidium labeled as upon the same host, proved to be upon *Solidago mollis* and the rust is described by Arthur, Bull. Torrey Club **31**: 7–8. 1904, as *Aecidium recedens*. See also Sydow, Monogr. Ured. **1**: 869. 1904.

UROMYCES INTRICATUS Cooke, Grev. **7**: 3. 1878.

Stated to be upon *Gayophytum ramosissimum*. The host, however, proves to be Eriogonum. See N. Amer. Fl. **7**: 244–245. 1912.

During the progress of this study, Professor Jackson obtained from the phanerogamic herbarium of the Field Museum, Chicago, a specimen (on Jussiaea sp., marsh land near Ferry River, vicinity of Kingston, Jamaica, Sept. 11–12, 1906, N. L. Britton, No. 397) which bears a very few small uredinial sori. The urediniospores are ellipsoid, 19–23 by 26–29 μ, the walls golden-brown, 1–2 μ thick, moderately echinulate, with equatorial pores. The known Jussiaea rusts are not believed to have an uredinial stage, but some Onagraceous rust, as for instance *Uromyces plumbarius*, may possibly occur upon Jussiaea.

The material is so scanty and the uncertainty so great, however, that one can only direct attention to this point.

As is to be expected, several genera of the Onagraceae are not known to be attacked by rusts. No other species of rust, than those herein listed, appear at present to be known to occur upon the Onagraceae.

As is stated in the beginning of this paper, the Sydows list 27 species of rust upon the Onagraceae, and 4 additional species occur in North America. Furthermore, the Sydows have not yet published the species *Aecidium Jussiaeae*, *Aecidium Circaeae*, and *Uredo oenothericola*. These 34 species are accounted for in this article under 17 titles. The arguments for this halving of the number of species are presented wherever a union is involved. Furthermore, a few other species are suggested as being of doubtful validity, notably numbers 16 and 17. It is suggested that the two races exist under the title *Pucciniastrum pustulatum* as herein treated.

Cultural data is necessarily of importance in limiting species and races; such data is at hand for but few of these rusts. The writer submits the foregoing arrangement of species, based upon a consideration of morphological characters and life histories, as well as hosts, distribution, and such limited cultural data as is at hand, in the hope that a workable arrangement may be presented. Finality of placement, is, of course, at the present time impossible.

The Onagraceae rusts, as far as the evidence in hand can be analyzed by the writer, appear, as partially indicated heretofore, to fall into three fairly definite groups, with an uncertain fourth group. (See the diagram.) The first is that of the Pucciniastrums, with the alternate stage, insofar as cultures have been successful, upon Abies. The morphological characters of the uredinial and telial stages upon Onagraceous hosts, are very similar within the different species of Pucciniastrum. They, however, can scarcely be construed to hint at any relation with the uredinial or telial stages of others of these rusts upon the same or similar hosts.

Following the suggestion of Dietel[10] that the Uredinales have developed during geologic times with their hosts, the Pucciniastrums would be the oldest of these rusts, since their aecial stage, so far as is known, occurs upon Gymnosperms. The telial stage further suggests a more primitive condition, resembling the fern rusts, which Dietel considers to be the oldest rusts. The genera Epilobium and Chamaenerion, the hosts of these species of Pucciniastrum, might therefore be considered to be older genera of the Onagraceae. Other facts, as indicated elsewhere in this article, point to the same conclusion. It is further to be noted that these two hosts harbor more species of rust than any other genera of the Onagraceae, including two heteroecious species, two autoecious long-cycled species, one with and one without uredinia, and three short-cycled species.

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[10] Dietel, P., Centr. Bakt., etc. **12**²: 218–234. 1904, and Hedwigia **48**: 118–125. 1908.

The second group is that including the long-cycled species *Uromyces plumbarius* and *Puccinia Epilobii-tetragoni*, and also the heteroecious species with its aecia upon the Onagraceae, *Puccinia Veratri*, the European species without uredinia, *Puccinia Epilobii-Fleischeri*, and the short-cycled species *Puccinia Epilobii* and *Puccinia scandica*. The morphological resemblances between the several species is so close that it seems quite logical to infer a relationship.

Uromyces plumbarius and *Puccinia Epilobii-tetragoni* differ but little in morphological characters in all the spore forms, save for the occurrence of one-celled and two-celled teliospores respectively. In other respects, however, the correlation does not hold as it does between certain other parallel species of Uromyces and Puccinia, for here a different set of hosts is attacked by the two species in question, and the geographical range of *Uromyces plumbarius*, while largely including that of *Puccinia Epilobii-tetragoni*, extends far beyond it, the Puccinia species being, in North America, wholly western, the Uromyces extending over the greater part of the United States. It is a curious fact that, so far as is known, the same species of host is not attacked by the two rusts. Indeed, unless they may meet upon the genera Oenothera, different host genera are attacked, that is, different genera as now subdivided. Twelve genera of the Onagraceae are given as hosts for *Puccinia Epilobii-tetragoni;* and seven genera as hosts for *Uromyces plumbarius;* while related genera obviously occur in the two sets, yet all are different. While deductions must be vague, this fact would seem at least to indicate that a rather definite and distinct specialization has arisen within these two species of rust. It might be inferred further that this specialization has occurred in a somewhat different way in each species; in the *Puccinia*, over a larger number of host genera, but limited geographically, in North America, to the west; in the Uromyces, over a greater area of North America, but limited to fewer hosts. *Uromyces plumbarius* also shows less variation than does *Puccinia Epilobii-tetragoni;* this is rather to be expected. An apparently complicating factor is that of the occurrence of this Puccinia upon various species of the genus Epilobium in some part of every continent, doubtfully excepting Africa. This is, however, in line with other relations of these rusts to the genus Epilobium, as is noted elsewhere. The variable American hosts are followed by variable rusts; this variability is further indicated by the fact that species of Uromyces occur only in America. Indirectly, it seems to the writer, the sharp difference in host genera attacked by the two above rusts reflects a considerable accuracy of taxonomic arrangement of hosts.

Puccinia luxurians and *Puccinia Krookii*, as stated, may be included with *Puccinia Epilobii-tetragoni.*

The relations of *Puccinia Veratri*, with aecia upon Epilobium, and of the other species of this group, has been indicated, and scarcely needs further comment. *Puccinia Veratri* has a more extended distribution than has *Puccinia Epilobii-tetragoni.* What conclusions are to be drawn from the comparatively greater frequency, but more limited distribution, of *Puccinia Epilobii-tetragoni*, as compared with the evident rarity, yet, in America, broader distribution of the short-cycled *Puccinia Epilobii* and *Puccinia scandica*, the writer is not prepared to say.

Into the third group may be placed *Puccinia Jussiaeae*, *Puccinia Circaeae*, *Puccinia gigantea*, *Puccinia Fuchsiae*, and *Puccinia Peckii.* The teliospores in this group are easily distinguishable from those of the group mentioned in the preceding paragraphs, being longer and narrower, often paler. Here, too, this similarity exhibited is close enough through all the species of this group to suggest a relation. *Puccinia Peckii* bears the same relation to this group that *Puccinia Veratri* does to the preceding group. As *Puccinia Veratri* is more extensive in range, in North America, than is *Puccinia Epilobii-tetragoni*, so *Puccinia Peckii* has also a more extended range than has *Puccinia Jussiaeae.* The hosts of the two latter species are in no cases identical, in spite of an evident relationship. This third group lacks, as far as known, a representative with uredinia upon the Onagraceae. While, of the short-cycled species in this group, *Puccinia gigantea* and *Puccinia Fuchsiae* appear to be more rare, *Puccinia Circaeae* is common.

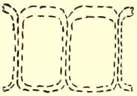
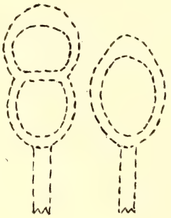
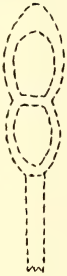
Into the fourth group must be placed the unknowns, *Aecidium Anograe*, *Aecidium Circaeae*, and *Uredo oenothericola*. (*Puccinia sphaeroidea*, as discussed previously, may be here omitted.) *Uredo oenothericola* may eventually land in the second group here given. Applying certain principles of correlation that have sometimes proven serviceable heretofore, one might vaguely prophesy regarding the alternate stages of *Aecidium Anograe* and *Aecidium Circaeae.* These two forms stand rather at extremes of the heteroecious aecial stages upon the Onagraceae. *Aecidium Anograe* has the largest aeciospores, and is the only one known possessing thick walls. *Aecidium Circaeae* has the smallest aeciospores, with thin walls. The former might be prophesied to go with an alternate form possessing rather large, thick-walled urediniospores; the latter, perhaps, with a form having small urediniospores.

Taking up the relation of these rusts to their hosts, a few points of interest are evident, in addition to those already presented in other connections. The related and cosmopolitan genera Chamaenerion and Epilobium harbor rusts that are placed in the first three groups just discussed. That there is some relation between the wide distribution, and, possibly, greater age of these genera, and the many, varied, and widely distributed rusts parasitic upon them, readily suggests itself. The most anomalous thing here appears to be the occurrence of the one representative of our third group, *i. e.*, *Puccinia gigantea.*

Another point with respect to hosts, rather out of harmony with expectations, is the conspicuous identity of the hosts of the different races of *Uromyces plumbarius* and those of the aecia of *Puccinia Peckii.* These two are not correlated species; and, indeed, as noted, neither of the two inhabits a host genus upon which an apparently correlated species does occur. The significance of this point seems perplexing to the writer.

Throughout these rusts upon the Onagraceae, wherever collections are in hand in sufficient numbers for a considerable comparison, variability is to be noted. Although short-cycled species are often found to be more constant, yet in the Onagraceae, the one American short-cycled species that is well represented in the Arthur herbarium has been found variable; it exists as a micro- or lepto-form in different seasons; the characters of the sori and teliospores vary. *Puccinia Jussiaeae* shows considerable variation. The marked variability of the autoecious species has been noted. One rather outstanding feature with

TABLE TO ILLUSTRATE THE GROUPS INTO WHICH THESE RUSTS FALL, WITH ALTERNATE HOSTS INDICATED WHERE KNOWN, AND DIAGRAMS OF THE GENERAL CHARACTER OF THE TELIOSPORES

1	2	3	4
Teliospores Adherent in Layers	Teliospores Free, Short-ellipsoid	Teliospores Free, Long-ellipsoid	(Probably Belong with Column 2 or 3, or Both)
			
II, III *Pucciniastrum pustulatum* (O and I unknown)	O, I, II, III *Uromyces plumbarius* *Puccinia Epilobii-tetragoni*	O, I, III *Puccinia Jussiaeae*	O, I *Aecidium Anograe* *Aecidium Circaeae*
(*P. Abieti-chamaenerii* O and I on Abies)	(O), I, III *Puccinia Epilobii-Fleischeri*	(O), III *Puccinia Circaeae* *Puccinia gigantea* *Puccinia Fuchsiae*	II *Uredo oenothericola* (Other stages of the above unknown)
Pucciniastrum Circaeae (O and I unknown)	(O), III *Puccinia Epilobii* *Puccinia scandica*		
	O, I *Puccinia Veratri* (II and III on Veratrum)	O, I *Puccinia Peckii* (II and III on Carex)	

regard to the variability of these rusts is the comparative constancy, in spite of wide distribution, of the several rusts upon the related genera Epilobium and Chamaenerion. This fact, with others, it seems to the writer, indicates that the variability of the rusts upon the Onagraceae, as similarly noted by Dr. Arthur with the rusts upon the Rosaceae, reflects the variability of the hosts themselves. Indeed, the evolution of these hosts and their rusts would appear to present many parallelisms.

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A STUDY OF PERMEABILITY BY THE METHOD OF TISSUE TENSION

S. C. Brooks

A number of investigators have shown that recovery from plasmolysis is not wholly satisfactory as a criterion of the permeability of protoplasm to a plasmolyzing agent because of the possible injurious mechanical effect of the plasmolysis itself.[1] It is evident that this difficulty disappears if instead of plasmolysis and recovery we use as a criterion the shrinkage and the elongation of young, highly stretched tissues, taking care that the shrinkage does not proceed far enough to cause a retraction of the protoplasm from the cell walls. This is most conveniently done with tissues in which the changes in turgidity are indicated by a bending of the tissue, due to great differences in the elasticity of the cell wall in its different layers.

In rapidly elongating plant tissues there is usually a very considerable pressure exerted by the protoplasts against the cell walls which confine them. If all the cell walls of the stem are thin and elastic, the whole stem will be kept in a stretched condition by this pressure. The presence of thick walled cells, such as fibro-vascular or epidermal cells, which are not easily stretched by internal pressure, will, if they are symmetrically distributed, prevent this elongation of the tissue. If now we cut such a stem or peduncle in such a way that these two types of tissue are unsymmetrically distributed, the whole tissue will curl so that the elastic tissue forms the longer, or convex side. The distension of the elastic tissues, and therefore the degree of curvature, will vary with the turgidity of the tissues. A hypertonic solution will withdraw water from the cells, and consequently reduce the turgidity and the degree of curvature, while a hypotonic solution will have the opposite effect. The penetration of the protoplasm by a salt with whose solution such a tissue had come into osmotic equilibrium would lead to an increase in the turgidity, and hence in the curvature of the tissue. De Vries (8), in the investigation of the isotonic coefficients of various substances by this method, observed such a secondary increase in the curvature of strips from the peduncles of *Centranthus ruber* and *Rudbeckia triloba.*

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[1] Cf. Bower (1), Chodat and Boubier (3), Hecht (4), and Küster (5).

Among such tissues, strips of peduncles of the dandelion (*Taraxacum officinale* Weber) are well known for their large and rapid response to changes in the concentration of the solution in which they are immersed. Such strips also showed themselves to be excellent material for the study of the rate of penetration of salts. Upon being cut, they are forced by the existing tension to bend rather strongly outward around an axis tangential to the peduncle. If they are then placed in a slightly hypertonic solution, their curvature decreases during a period varying from a few seconds up to several minutes, remains constant a moment and then slowly increases, sooner or later exceeding the original curvature. The last phase is analogous to the recovery of plasmolyzed cells, and will, for convenience, be also termed "recovery."

By means of observations on the rate at which this recovery occurred in various salt solutions it was possible to determine the permeability of dandelion protoplasm to inorganic salts, and the progressive changes in permeability produced by such salts.

METHOD

The salts used were the same or of the same grade of purity as those used in the experiments on exosmosis from dandelion tissue, as described by the writer in a recent paper (2). They were dissolved in distilled water which had a specific conductivity of less than 2×10^{-6} ohms. Molecular stock solutions were made up and carefully standardized by titration against a 0.1 *M* solution of silver nitrate with potassium monochromate as an indicator. The desired concentrations were secured by dilution of these stock solutions, and were accurate to 0.5 percent. This accuracy was sufficient for the purposes of the experiment.

Strips of dandelion peduncle, each about 2.5 cm. long and 3 mm. wide, or similar strips from the midrib of the leaf were used in these experiments.[2] There was practically no difference in the data furnished by the two types of tissue.

Each strip was firmly gripped at one end by the two halves of a partially split rubber stopper, which in turn was secured by means of

[2] The midrib, like the peduncle, is hollow and for the sake of uniformity strips from the upper (ventral) half of this tissue were always used.

de Khotinsky's cement to the bottom of a glass dish 6.5 cm. in diameter and 1.5 cm. deep. The strips were held horizontally in such a position that as the free end bent it moved back and forth horizontally.

Immediately after the strips of peduncle were cut, they were put in place in the glass dish and covered with 20 cc. of an isotonic solution of the salt to be investigated. The dish was then covered with a glass plate and set on the stage of a microscope. The glass plate was pierced by an opening just large enough to allow the introduction of the front of the Bausch and Lomb 2/3 inch objective, by means of which, in combination with a No. 7.5 ocular, provided with an ocular micrometer, the position and rate of movement of the free end of the strip was determined. Figure 1 shows the arrangement of the apparatus.

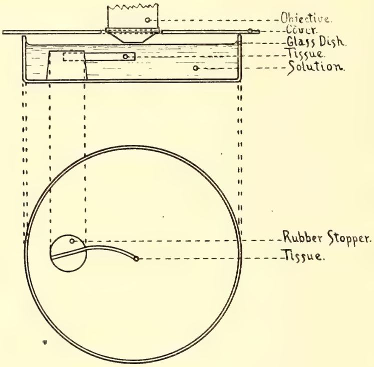

FIG. 1.

The epidermal surface of the strip furnished a sharply defined point of reference. The evaporation from a dish so covered, with the objective in place, amounted to about 0.05 percent per hour at room temperature; this amount was not sufficient to have any appreciable effect on the time required for recovery.

A solution was considered to be isotonic with the cells of the tissue when there was a barely perceptible decrease in the curvature of the strips of peduncle immediately following their immersion in the solution. This condition signified that the solution was actually slightly hypertonic to the cells; but a very slight shrinkage of the cells, such as caused the decrease in curvature of the tissue, sufficed to raise their osmotic pressure to equality with that of the solution, and recovery proceeded as rapidly as the rate of penetration of the plasmolyzing substance would permit. It will be seen that while the solutions used were not strictly isotonic with the cells, the latter adjusted themselves to the solution, becoming isotonic with it within at most a very few minutes.

As soon as a condition of isotony was established between cells and solution, the concentration of the latter was increased by the addition of a measured small amount of a molecular solution of the salt, and the solution quickly stirred in order to secure a uniform distribution of the increase in concentration. In order to avoid very great differences in the amount of shrinkage, such as might cause a mechanical alteration of the cell walls, or injury to the protoplasm, it was necessary to vary this increase in concentration according to the salt used. Thus, for the sea water-calcium chloride mixture, described by the writer in a previous paper (2), and for the salts of univalent kations 0.2 cc. of 1 *M* solution was added to 20 cc. of the isotonic solution which had a concentration of 0.20 to 0.235 *M*. In the case of bivalent kations the concentrations were 0.15 to 0.17 *M* and to 20 cc. of the solution there was added 0.1 cc. of a 1 *M* solution to produce the desired increase of concentration. In the case of trivalent kations the concentrations were .045 to .065 *M* and 0.05 cc. of a 1 *M* solution was added when it was desired to increase the concentration. The maximum error in making these changes was 0.0001 *M*.

This increase in concentration resulted in a decrease in the curvature of the strip of tissue which soon ceased and was followed by a slower movement in the opposite direction. The time elapsing between the increase of concentration and the moment when the strip regained its original curvature (*i. e.*, returned to its initial position on the scale of the ocular micrometer) was recorded as the "time of recovery." Immediately upon the recovery of a given strip, the concentration of the solution bathing it was again raised by the same amount as before, and the time of recovery again noted. By repetition of this process we secure a series of recoveries of one strip of tissue, the time required for each recovery being a measure of the average rate of penetration of salt during that recovery, and the initial and final curvature being always the same throughout the whole of the experiment.

In order to obtain figures for the different groups of salts which would be comparable with one another, it was necessary to correct for the difference between the concentration changes used for these groups. An empirical expression of the rate of penetration of a salt may be obtained by dividing the concentration change causing the decrease in curvature by the time in minutes required to regain the initial curvature, or, as we have termed it, the "recovery time."

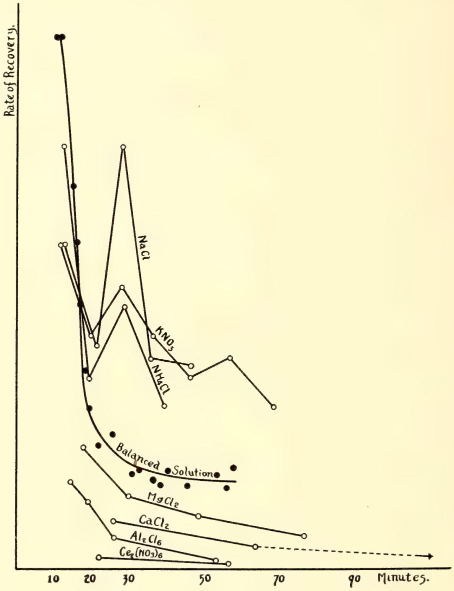

FIG. 2

If such figures be used as the ordinates, and as abscissae the time elapsed between the first immersion of the tissue in the solution and the middle of the recovery time, there will be produced a curve expressing the permeability of the cells after any given period of exposure to the action of a salt. Such curves are shown in Figure 2.

RESULTS

It was found that in all the solutions the rate of recovery decreased rapidly during the first fifteen or twenty minutes, and that all the experiments, with the exception of those with the sea water—calcium chloride solution, gave data which were quite erratic during that period. An examination of the curve (Fig. 2) representing the rate of penetration of this mixed solution will show that the points, plotted from four experiments, fall very uniformly on a smooth curve which shows a rapid decrease in the rate of recovery during the first twenty-five minutes, after which the rate remains nearly constant. This decrease in the rate of recovery represents the effect of some factor whose nature has yet to be determined, and which is common to all the salts used. In view of the presence of this factor and of the erratic behavior of the tissue during the first one or two recoveries in solutions other than the sea water-calcium chloride mixture, it seems best, for the present, to disregard the first twenty minutes of these experiments.

TABLE I

Time Required for Successive Recoveries, Taraxacum

Kations	Solution	Concentration	Increase of Concentration	Minutes in Solution Before 1st Recovery	Recovery Time, Minutes						
					1st	2nd	3d	4th	5th	6th	7th
Balanced	Sea water and $CaCl_2$ Mixture	0.235	0.0075	9	4	13	25	—	—	—	—
				10	4	16	21	—	—	—	—
				10	2.5	5.5	15.5	25	—	—	—
				13	10.5	24	21	—	—	—	—
				13	8	21.5	22	—	—	—	—
				13	6.5	23.5	26	—	—	—	—
Univalent	$NaCl$	0.22	0.0075	10	6.5	9.5	5.0	10	10.5	—	—
	KNO_3	0.21	0.0075	9	6.5	9.0	7.5	9.0	11.0	10.0	13.0
	NH_4CL	0.23	0.0075	6	3	5	11	8	13	—	—
Bivalent	$CaCl_2$	0.165	0.0041	14	24	51	83	—	—	—	—
	$MgCl_2$	0.15	0.0041	13	9.5	15	22	34	—	—	—
Trivalent	$Ce_2(NO_3)_6$	0.065	0.0011	9	26	43	—	—	—	—	—
	Al_2Cl_6	0.047	0.0011	13	3.5	4.5	9.5	—	45	—	—

In the experiments with solutions of pure salts the time at which characteristic alteration of permeability occurred varied sufficiently with different peduncles and even with adjacent strips from the same peduncle, to make it ordinarily impossible to construct a composite curve which should fairly represent the characteristic effect of the salt. Therefore experiments have been selected which represent as nearly as possible the mean of all the experiments with the same salt, and the curves representing the progressive changes in rate of recovery plotted in Figure 2. The original data of these experiments are given in Table 1.

The difference in the behavior of the three groups of kations employed is very striking. The curves for sodium, potassium and ammonium salts lie everywhere above those for the mixed solution; the rate of recovery, and therefore the rate of penetration of the salt, is and remains greater than that normal for the protoplasm. The rate of penetration of calcium and magnesium salts is considerably, and that of salts of the trivalent kations (cerium and aluminium) very much below the normal.[4] It is to be noticed that sodium, potassium, and ammonium salts cause a marked increase in the rate of recovery between the twentieth and thirtieth minutes.

The secondary decrease, in the light of the experiments on the influence of salts on exosmosis, is probably to be attributed to the fact that the increase of permeability leads to a considerable rate of exosmosis, thus retarding the increase of the intracellular osmotic pressure, and hence decreasing the rate of recovery. It will be seen that sodium and ammonium chlorides, the most toxic among the three salts of univalent kations, cause the most rapid secondary fall of the curve, while potassium nitrate, the least toxic, causes only a slight fall. This fact also favors the supposition that the cause of secondary retardation in the rate of recovery is to be sought in an increase rather than a decrease of permeability; but this explanation must remain purely hypothetical pending the accumulation of further evidence with respect to the phenomenon. These experiments are thus found to be in essential agreement with those of Osterhout in which *Laminaria* was the plant used, although the fluctuations in permeability induced by pure salts were greater and more rapid than those in *Laminaria*. The

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[3] The first recovery times are, however, usually in the same sequence in a series of salts as are the later recoveries.

[4] These curves were probably not disturbed by differences in the acidity of the solutions, except possibly in the case of cerium and aluminium chlorides, whose hydrogen ion concentrations, as determined by the use of a hydrogen electrode, were $3 \times 10^{-4} M$ and $4 \times 10^{-4} M$ at the concentrations used.

writer's experiments on *Laminaria*, by a diffusion method, are also in essential agreement with these experiments.[5]

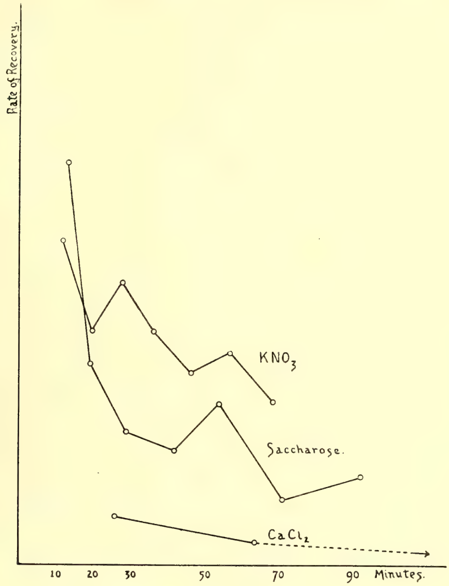

FIG. 3

TABLE 2

Time Required for Successive Recoveries, Taraxacum

Solution	Concentration	Increase of Concentration	Minutes in Solution Before 1st Recovery	Recovery Time, Minutes						
				1st	2d	3d	4th	5th	6th	7th
KNO_3......	0.21	0.0075	9	6.5	9.0	7.5	9.0	11.0	10.0	13.0
$CaCl_2$......	0.165	0.0041	14	24	51	83	—	—	—	—
Saccharose .	0.35	0.0064	11	5	8	12	14	10	24	18

In view of the widespread assumption that protoplasm is in general impermeable to saccharose, and that cells may be kept in solutions of saccharose without suffering any change in permeability,[6] the data of experiments on the rate of recovery in saccharose solutions are of considerable importance. In Table 2 and Figure 3 are presented the data of a characteristic experiment with saccharose, together with those of similar experiments with potassium nitrate and calcium chloride for purposes of comparison. Saccharose affects the permeability of the protoplasm in the manner typical of a salt of a monovalent kation, but the changes occur with less rapidity. It is therefore unsafe to use saccharose as an indifferent medium; a properly balanced mixture of salts should rather be used when a solution is desired in which to maintain the normal permeability of living cells, as is evident from a comparison of Figures 2 and 3, disregarding (as previously explained) the first 20 minutes of the experiment.

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[5] In process of publication.

[6] Cf. Lepsechkin (6).

SUMMARY

1. The permeability of the protoplasm of *Taraxacum officinale* Weber remains nearly or quite normal in a balanced solution consisting of a mixture of sea water and calcium chloride, such that the ratio of univalent to bivalent kations is approximately seventy to fifteen.
2. Salts of univalent kations in pure solutions cause a rapid increase in permeability.
3. Salts of bi- and trivalent kations cause a very great decrease in permeability.
4. Saccharose penetrates protoplasm quite rapidly, and affects permeability like a univalent kation.

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OSBECK'S DAGBOK ÖFWER EN OSTINDSK RESA

E. D. MERRILL

Pehr Osbeck, according to Pritzel, was born at Oset, Sweden, May 9, 1723, and died at Hasslof, Sweden, December 23, 1805. He had attended the University of Upsala, where he had received instruction in natural history from Linnaeus. His rather important contribution to our knowledge of the Chinese flora has quite consistently been overlooked by most botanists. We find few references to species described by Osbeck, while a number of the forms he named and described are not listed in Index Kewensis. His name is perpetuated, however, by the genus *Osbeckia*, named in his honor by Linnaeus, while the Linnaean herbarium contains about 600 specimens of plants, from China, Java, and Spain, collected by him.[1] A number of species included by Linnaeus in the first edition of his Species Plantarum are based on Osbeck's specimens, while others are included in later works of this author. Usually the names given by Linnaeus are the same as those used by Osbeck, although rarely is Osbeck cited as the author of a species, while a number of plants characterized and named by him were ignored by Linnaeus.

In his "Dagbok" Osbeck mentions nearly 500 species of plants, of which about 80 are accompanied by descriptions in Latin. Twenty-six species and two genera are named and described for the first time, but few of them are included in any subsequent botanical work. About twenty *nomina nuda* also appear in the book.

Osbeck left Sweden November 18, 1750, as chaplain of the "Prince Charles," one of the ships belonging to the Swedish East India Company, bound for Canton, China, and returned June 26, 1752. En route he gave much attention to the study of natural history, and described not only plants, but also birds, mammals, reptiles, fishes, and insects. His contributions to botany apply mainly to the floras of Spain, Java, and southern China, mostly to the latter; his stop in Java was brief. He remained with his ship in the vicinity of Whampoa and Canton, China, from August 23, 1751 to January 4, 1752.

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[1] Jackson, B. D. Index to the Linnaean Herbarium 16, 1912.

The full title of Osbeck's work is as follows: "Dagbok | Öfwer | en | Ostindsk | Resa | Åren 1750. 1751. 1752. | Med Anmärkningar | Uti | Naturkunnigheten, främ- | mande Folkslags Språk, Seder, | Hushållning, m.m. | På Fleras åstundan | Utgifwen | Af | Pehr Osbeck. | Kongl. Sw. Wettenskaps Societetens Ledamot. | Jämte 12 Tabeller | Och Afledne Skepps-Predikanten Toréns Bref. | Stockholm | — | 1757." The text is in Swedish, but where plants and animals are described the descriptions are usually in Latin. The book consists of a short introduction, three hundred and seventy-six pages of text, an index, and twelve plates. A copy of this rare work is in the library of the Arnold Arboretum, and a photographic reproduction of it is in the library of the United States Department of Agriculture.

In the year 1765 a German translation was published by J. G. Georgi under the title: "Herrn Peter Osbeck | Pastors zu Hasslöf und Woxtorp, der Königl. Schwedischen | Akademie zu Stockholm und der Kön. Gesellschaft zu Upsala, | Mitgliedes | Reise | nach Ostindien und China. | Nebst O. Toreens Reise nach Suratte | und C. G. Ekebergs Nachricht von der Landwirthschaft | der Chineser. | Aus dem Schwedischen Übersetz | von | J. G. Georgi | Mit 13 Kupfertafeln." This consists of an introduction of twenty-four pages, 552 pages of text, index, and thirteen plates. The descriptions of plants and animals, in Latin in the original edition, are here translated into German.

In the year 1771 J. R. Forster translated the German edition into English, under the following title: "A | Voyage | to | China and the East Indies, | By Peter Osbeck, | Rector of Hasloef and Woxtorp, | Member of the Academy of Stockholm, and of the | Society of Upsal. | Together with a Voyage to Suratte, | By Olof Toreen, | Chaplain of the Gothic Lion East Indiaman. | and | An Account of the Chinese Husbandry, | By Captain Charles Augustus Eckeberg. | Translated from the German, By John Reinhold Forster, F. A. S. | To which are added, A Faunula and Flora Sinensis." This was issued in two volumes. **1** (1771) V+1–396, *pl. 1–13;* **2** (1771) 1–367, index. This edition is very rarely cited in botanical literature as Osbeck's "Iter ed Angl." It is of special interest because it contains the first attempt to enumerate, in systematic order, the species of the Chinese flora after the establishment of the binomial system of nomenclature. Pages 339 to 367 are devoted to this enumeration, apparently the work of the translator, under the title "Flora Sinensis: or, An Essay towards a Catalogue of Chinese Plants." About 260 species are listed, compiled from the writings of Linnaeus and Osbeck. Copies of German and English editions are in the Library of Congress, Washington. Bretschneider[2] under the heading "Swedish collectors of plants in South-China," 1751 and 1766, pp. 88–119, discusses the work of Osbeck, Toren, Eckeberg, Sparrman, and Lagerstroem in connection with the early botanical exploration of southern China, and enumerates 319 species of plants that were known from this region by Linnaeus. His data regarding Osbeck were taken from Forster's English translation of Osbeck's work.

The species described by Osbeck from Java were collected at or near sea level, at Anjer and New Bay, at the west end of the Island. Those described from China were chiefly from the vicinity of Whampoa with a few from Canton. In many cases the descriptions are quite sufficient to enable one accurately to determine the species Osbeck intended, even when the descriptions are relatively short. Occasionally, however, I have been unable to determine the status of a species. Many are represented by actual specimens in the Linnaean herbarium in London. Wherever it has been possible to determine the status of Osbeck's species, his specific names, when valid, have been adopted even when they displace names of later authors now in common use. In a few cases Osbeck's name must displace that of Linnaeus as the authority for various species, where there is no change in the specific name. A slight amount of field work, or perhaps a more intensive knowledge of the flora of Kwangtung than I possess may enable future investigators more exactly to determine the status of the few remaining doubtful species.

I am in grave doubt as to the propriety of adopting certain specific names from Osbeck, as Swingle has recently proposed.[3] In these cases the Latin name appears, but there is, usually, no description in Latin, while the statements in Swedish regarding the fruits or the plants, can hardly be considered as descriptions. The special cases are *Rhamnus thea* Osbeck, *Citrus grandis* Osbeck, *C. sinensis* Osbeck, and perhaps *C. limonia* Osbeck. At any rate it is doubtful if we are warranted in displacing a specific name of a later author on the basis of the meager data given by Osbeck in the case of the above species.

In addition to the valid corrections and additions to Index Kewensis, a considerable number of *nomina nuda* occur which in Osbeck's work are here enumerated merely to call attention to the fact that they are *nomina nuda*. None of them occur in Index Kewensis. The list is as follows: *Abies chinensis*, p. 217; *Ammi hispanicum*, p. 57; *Buxoides aculeata*, p. 242; *Calla javanica*, p. 277; *Cacalia incana*, p. 234; *Cucurbita chinensis*, p. 292; *Curcuma chinensis*, p. 205; *Cyperus dichotomus*, p. 229; *Gratiola virginianoides*, p. 205; *Nyctanthes orientalis*, p. 136; *Rhus chinensis*, p. 232; *Saccharum pluviale*, p. 130; *Salicornia fruticosa*, p. 56; *Stachys hirta*, p. 46; *Stachys arvensis*, p. 47; *Tillaea procumbens*, p. 57; *Toxicaria rumphii*, p. 90. Other *nomina nuda* in groups not covered by Index Kewensis are *Lichen chinensis*, *Jungermannia chinensis*, *Hypnum javanense*, and *Lycopodium varium*.

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[2] Early European Researches into the Flora of China. Journ. North-China Branch Roy. As. Soc. II, **15**: 1–194. 1880.

[3] Sargent, Plantae Wilsonianae **2**: 141–151. 1914.

A few slight differences in names, due to typographical errors, are noted, such as *Smilax sassaparilla*, p. 248, instead of *S. sarsaparilla; Hypochoeris radiata*, p. 48, instead of *H. radicata; Jasminum azoreum*, p. 272, instead of *J. azoricum;* and *Scorpiurus falcata*, p. 73, German edition, instead of *S. sulcata* as it correctly appears in the original edition.

Generic names originated by Osbeck are *Cryptanthus*, *Tetradapa*, and the *nomina nuda Buxoides* and *Toxicaria*, none of which have been used by subsequent authors in Osbeck's sense. They are not listed in any subsequent botanical work.

Below are listed the species described by Osbeck that have been overlooked by most botanists. New combinations are made in a few cases. The original descriptions are quoted in full, and, where essential, an English translation of Osbeck's Swedish description or comment is added. At the end is given a summary of the species not listed in Index Kewensis, and those listed, but their places of publication erroneously cited; the *nomina nuda* are mentioned above.

I am indebted to Mr. Ivar Tidestrom of the U. S. Department of Agriculture for direct translation of some of Osbeck's comments and descriptions from the original Swedish, for comparison with the published German and English translations.

Achyranthes chinensis Osbeck, Dagbok Ostind. Resa 205. 1757 = ? *A. aspera* L.

This is probably a synonym of *Achyranthes aspera* L., or *A. bidentata* Blume, if placed by Osbeck in the right genus. The description is inadequate, and apparently bracts or bracteoles were interpreted by the author as sepals. The original description is as follows: "*Achyranthes chinensis.* Obs. Perianthum duplex, corolla longius. Corollam 5 petalum includens; exterius minus, diphyllum, interius 5 phyllum. Flores racemosi, terminatrices. Pedunculi ex alis. Folia lanceolata, opposita, nervosa, glabra. Caulis ruber." This plant was observed September 8, 1751, near Canton, and while the description does not entirely agree with *Achyranthes aspera* or *A. bidentata*, it applies better to these species than to any other amaranthaceous plant known from Kwangtung Province, China.

Agaricus chinensis Osbeck, Dagbok Ostind. Resa 221. 1759.

The description, which is entirely inadequate, is as follows: *Agaricus* (*chinensis*) stipite albo, spithameo, pileo lutescente." The plant was from the vicinity of Whampoa, September 12, 1751.

Albizzia chinensis (Osbeck) comb. nov.

Mimosa chinensis Osbeck, Dagbok Ostind. Resa 233. 1757.
Mimosa marginata Lam. Encycl. **1**: 12. 1783.
Mimosa stipulata Roxb. Hort. Beng. 40. 1814, *nomen;* Fl. Ind. ed. 2, **2**: 549. 1832 (*stipulacea*).
Acacia marginata Hamilt. in Wall. Cat. No. 5243. 1832.
Albizzia stipulata Boiv. Encycl. XIX Siècle **2**: 33. 1838.
Albizzia marginata Merr. in Philip. Journ. Sci. Bot. **5**: 23. 1910.

Osbeck's *Mimosa chinensis* has been overlooked by all botanists, and is not listed in Index Kewensis. The description is good, and applies unmistakably and exclusively to the species generally known as *Albizzia stipulata* Boiv. The original description is here given: "*Mimosa* (*chinensis*) inermis, stipulis foliolo longe majoribus, semicordatis. *Folia* septem vel octojuga. *Foliola* numerosa, fere lanceolata, sed basi obtusiora. Differt imprimis & manifeste a reliquis maximis suis stipulis, quae semicordatae cauli adsident eumque amplectuntur, plusquam decies majores foliolis. Flores non vidi." The type was from near Whampoa, where the plant was observed by Osbeck, October 6, 1751. Among all the Asiatic *Mimosoideae* Osbeck's description applies only to *Albizzia stipulata* Boiv. It grows in southern China, is rather widely distributed in southeastern Asia, and is also found in the Philippine Islands, in Java, probably in other islands of the Malay Archipelago, and in the Andaman and Nicobar Islands.

Briza elegans Osbeck, Dagbok Ostind. Resa 246. 1757 = ? *Eragrostis elegantula* Steud.

The original description of this species is as follows: "*Briza* (*elegans*?) spiculis oblongis, valvulis carinatis" followed by a statement in Swedish to the effect that it was a very beautiful grass that grew at the highest plantations. It was from Dane's Island, near Whampoa, October 24, 1751.

Rendle[4] includes this species under *Briza*, but states that he had seen no specimens; he suggests that it might be a synonym of *Eragrostis major* Host. Dunn & Tutcher[5] also enumerate it, with the comment: "Near Canton. Fl. April to June." In both publications the original place of publication is cited correctly, but the species is not included in Index Kewensis. *Briza* is known from China by but a single species, *Briza minor* L., this apparently introduced, and northern in range (Province of Chihli). No representative of the genus is known from southern China. I am of the opinion that *Briza elegans* Osbeck is probably a synonym of *Eragrostis elegantula* Steud.; at least that it is an *Eragrostis*, certainly not a *Briza*. The specific name in *Eragrostis* is invalidated by *Eragrostis elegans* Nees.

Ceratolobus javanicus (Osbeck) comb. nov.

Caryota javanica Osbeck, Dagbok Ostind. Resa 270. 1757.

Ceratolobus glaucescens Blume in Roem. & Schult. Syst. Veg. **7**: 1334. 1829.

This species has been overlooked by all botanists and is not enumerated in Index Kewensis. Osbeck's specimen was from New Bay, Java, where the plants were observed January 19, 1752. The original description is here repeated: "*Caryota* (*javanica*) frondibus bipinnatis, aculeatis; foliolis cuneiformibus, rotundato-praemorsis. Fructificationem non vidi; unde genus obscurum. Frondes sunt bipinnatae, ut in *Caryota*, subtus albidae. Pinnae alternae, obovatae, plicatae, margine superiori rotundato inaequalis. Petioli superne aculeis plurimis oppositis, recurvatis, non solum ad pinnarum exortum, sed etjam saepe par enum vel 2 inter pinnas." The description is introduced by a statement in Swedish, of which the following is a translation: "A great number of palms 6 to 12 feet high, with curved thorns on the leaves, held us fast and would not let go until our clothes were almost torn asunder, as well as the skin on the hands and the face." This description applies unmistakably to a group of palms, the rattans, characteristic of the Indo-Malayan region, the " prehensile " characters of which are well known to all who have traveled in the forests of the Malay Archipelago. Among all the Javan species, however, Osbeck's description applies best to *Ceratolobus glaucescens* Blume, the only other possible species being *Korthalsia robusta* Blume. However, Osbeck's specimen was from near sea level in western Java, which practically excludes the *Korthalsia* from consideration in this connection. Koorders[6] gives the distribution of *Ceratolobus* as "West Java: In den unteren Gebirgsgegenden von den Residenzschaften Bantam, Batavia, und Preanger . . .," while, according to the same author, the occurrence of *Korthalsia robusta* Blume in western Java is doubtful.

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[4] Journ. Linn. Soc. Bot. 36: 421. 1904.

[5] Fl. Kwangtung and Hongkong 329. 1912.

It is to be noted that Osbeck describes the leaves of *Caryota javanica* as *bipinnate*, which is not true of any of the Malayan palms that are supplied with hooked spines. The term *bipinnate* was apparently used to make the description of *Caryota javanica* agree with the generic description of *Caryota*, through faulty observation, or possibly because the exact significance of the term was not understood by the author. It is apparent, however, that Osbeck prepared no botanical material of this species, and that he probably wrote up his diagnosis, at least in part, from memory.

Catesbaea ? **javanica** Osbeck, Dagbok Ostind. Resa 92. 1757 = *Clerodendron commersonii* (Poir.) Spreng. (*C. inerme* Auct., haud Gaertn.)

Osbeck's species is not listed in Index Kewensis, and his specific name, although much older than *Volkameria commersonii* Poir. (1808), is invalidated in *Clerodendron* by *C. javanicum* Spreng. The species is the common coastal form that has been called by most botanists *Clerodendron inerme* (L.) Gaertn., but which Clarke[7] and Gamble[8] have retained as a distinct species under the name *Clerodendron neriifolium* Wall. (1829). Osbeck's type was from near Anjer, Java, observed by him July 16, 1751, associated with *Acanthus ilicifolius* L., *Convolvulus pes-caprae* L., *Ischaemum muticum* L., *Vitex trifolia* L., and other typical strand plants. The original de-

[6] Exkursionsflora von Java **1**: 226. 1911.

[7] Hook, f. Fl. Brit. Ind. **4**: 589. 1885.

[8] Journ. As. Soc. Bengal **74**^{2}: 827. 1909.

scription is as follows: "*Catesbaea ? javanica.* Descr. Calyx: Perianthium infundibuliforme, brevissimum. Corollae tubus longissimus, subcylindraceus: Limbus brevis, quinquepatitus. Filamenta 4 filiformia, longissima, corollae tubo inserta. Antherae parvae. Germen rotundum parvum. Stylus filiformis, staminibus longior. Flores caerulei ex alis; pedunculi ad summum triflori: pedicelli breves. Frutex Rami penduli quadrangulares. Folia ovato-lanceolata opposita glabra acuta petiolata secunda decidua. Habitat ad mare." This description agrees closely with *Clerodendron commersonii*, and there is, moreover, no other known Javan coastal plant to which it at all applies.

Citrus grandis Osbeck, Dagbok Ostind. Resa 98. 1757.

This is scarcely more than a *nomen nudum*, yet there is no doubt whatever as to the identity of the species intended. Swingle[9] has adopted Osbeck's name in place of *Citrus decomana* L. There is no Latin description, and no reference to *Citrus aurantium* L. var. *grandis* L. Sp. Pl. 783. 1753. Under the heading " Pompelmoser " Osbeck describes two forms or species, the first the common pomelo as *Citrus grandis*, the second the form that the Javanese called *pompelmus*. The following is a translation of Osbeck's statement: " Pompelmoser, *Citrus grandis* is a large round fruit resembling an orange but much larger, and on account of its acidity more refreshing than the orange, in the place of which it is eaten after meals. The rind is spongy, as thick as one's finger and bitter as in the *pomeranser* (the bitter or Seville orange), to which this fruit is nearest related. There was another kind which the Javanese called *pompelmus*, a round fruit like a small orange but green and covered with green warts. Of this I saw but two. It was more expensive, sweeter and better flavored than the preceding."

Under the latter Osbeck gives, with doubt, a footnote reference to " Limon tuberosus martinicus, malice Lemon martin. Rumph. 2. p. 101. t. 26? " The oldest binomial for the pomelo is *Aurantium maximum* Burm. Index Universalis Herb. Amb. 16. 1755.

Citrus limonia Osbeck, Reise Ostind. China 250. 1765.

Swingle[10] has adopted Osbeck's name for the form generally known as *Citrus limonum* Risso or *C. medica* var. *limonum* Hook. f., the common lemon. As noted by Swingle the specific name does not appear in the original (1757) Swedish edition of Osbeck's work, but does in the German (1765) edition. The original description, in Latin, appears as a footnote from the Chinese name *Lam-tjes* on page 192 or Osbeck's Dagbok, and is as follows: "Caulis teres, subscaber, cinereus, pallide striatus. Rami inordinati, patentes, reflexi, rarius spinosi. Ramuli spinis rectis, acutissimis, alternis vel ex alis ramulorum. Folia alterna, oblongo-lanceolata, petiolata, subcrenata. Petioli subalati, lineares." There is a discussion in Swedish to the effect that the Chinese make punch from the unripe fruits that they sell to the sailors, and that the plants are grown in pots for sale.

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[9] Sargent, Plantae Wilsonianae **2**: 144. 1914.

[10] Sargent, Plantae Wilsonianae **2**: 146. 1914.

Citrus sinensis Osbeck, Dagbok Ostind. Resa 41. 1757, *nomen;* Reise Ostind. China 250. 1765.

Swingle[11] has adopted Osbeck's name for the sweet orange which he considers to be specifically distinct from the sour orange (*Citrus aurantium* L.). There is no description in the original Swedish edition of Osbeck's work, and in the German edition (1765) is given a very inadequate description which includes also the mandarin orange (*Citrus nobilis* Lour.).

Clematis chinensis Osbeck, Dagbok Ostind. Resa 205, 242. 1757.

Osbeck's species should not be confused with *Clematis chinensis* Retz., which is apparently an entirely different form. It is probable that *Clematis meyeniana* Walp. is a synonym of *Clematis chinensis* Osbeck. The original description, on page 205, is as follows: "*Clematis chinensis.* Obs. Plurima habet cum Clematide Vitalba, at folia lanceolata, angustissima, & flores minores," while on page 242, the following additional data are given: "Obs. pistilla 3 ad 6, stylis plumosis in orbem positis, reflexis. Stam. O obseruavi. Frutex scandens, ramosissimus." In Index Kewensis the species is included, but the place of publication is given as the 1771 English edition "*Clematis chinensis* Osbeck Iter. ed. Angl. 1, 392"; here it is erroneously reduced to the European *Clematis recta* L. Osbeck observed the species in the neighborhood of Canton September 8, and again near Whampoa, October 20, 1751. Nine species of the genus are known from Kwangtung Province, but considering the characters given by Osbeck, and the distribution of the various species, the probabilities are very great that *Clematis meyeniana* Walp. is the species intended by Osbeck.

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[11] Sargent, Plantae Wilsonianae **2**: 148. 1914.

Commelina chinensis Osbeck, Dagbok Ostind. Resa 242. 1757 = *Commelina nudiflora* L.

There is little doubt as to the correctness of this reduction. The original description is as follows: "*Commelina chinensis.* Chinensibus Ka-tjaa. Corolla aequalis; Caulis nodosus. Folia lineari-lanceolata, alterna, vaginantia, villosa, an Commelina nudiflora?" The description of the leaves, as to shape, applies sufficiently well to the Linnaean species, but they are scarcely villous, although sometimes sparingly pubescent. The specimen was collected at Dane's Island, near Whampoa, October 20, 1751. The species is not listed in Index Kewensis.

Convallaria chinensis Osbeck, Dagbok Ostind. Resa 220. 1757 = ? *Scilla chinensis* Benth.

This is probably the same as *Scilla chinensis* Benth. Fl. Hongk. 373. 1861, but Bentham's specific name was not based on Osbeck's. The original description, which is entirely inadequate, is as follows: "*Convallaria* (*chinensis*) foliis linearibus, corollis sexpartitis. Obs. Est. quasi inter Scillas & Convallarias." The specimens were from ' Frans " (= French) Island, near Whampoa, September 13, 1751.

Cryptanthus chinensis Osbeck, Dagbok Ostind. Resa 215. 1757 = ?

The Latin description of this new genus and species consists of four words only in the form of a footnote: "Foliis oppositis, Rubi facie." A short description is given in Swedish, of which the following is a translation: " In the direction of the city there grew a kind of small bush, about as high as gooseberry bushes, with double white flowers. The leaves are as large as those of the rose mallow, cordate, blunt-serrate, the margins with unequal lobes, pubescent on the upper surface, smooth beneath and with at least eight primary nerves, the flowers in terminal racemes."

The description alone is insufficient to identify this plant. The genus, which has escaped the attention of all botanists, is entirely different from *Cryptanthus* Otto & Dietr. (1836), *Cryptanthus* Nutt. (1849), and *Cryptantha* Lehm. (1836). The plant was from Dane's Island, near Whampoa, September 11, 1751.

Desmodium styracifolium (Osbeck) comb. nov.

Hedysarum styracifolium Osbeck, Dagbok Ostind. Resa 247. 1757; L. Syst. Veg. ed. 10. 1169. 1759.

This species has been reduced to *Desmodium retroflexum* (L.) DC., but *Hedysarum styracifolium* Osbeck is an older name than *H. retroflexum* L., on which DeCandolle's species is based. The type was from Dane's Island, near Whampoa, October 25, 1751, and the original description is as follows: "*Hedysarum* (*styracifolium*) foliis simplicibus cordato-orbiculatis, retusis, supra glabris."

Fucus maximus Osbeck, Dagbok Ostind. Resa 283. 1757 = *Ecklonia buccinalis* (L.) Hornem.

This was from latitude 33° 13′ S. in the vicinity of Cape Colony, March 10. The original description, presented as a footnote under "Trumpet-grass," is as follows: "*Fucus* (maximus) caule tereti, fistuloso, simplici, flabello quasi terminato. An Fucus pavonicus ? cfr. Trombas. G.M.A.V.V.L. Descriptio itin. navalis in ind. p. 51. fig. mala. Obs. Folia terminalia, adgregata, distica, inferiora gradatim minora. Caulis defoliatis." Osbeck's specific name antedates the Linnean *Fucus buccinalis* on which *Ecklonia buccinalis* is based, but no change is here made.

Limnophila chinensis (Osbeck) comb. nov.

Columnea ? chinensis Osbeck, Dagbok Ostind. Resa 230. 1751.
Limnophila hirsuta Benth. in DC. Prodr. **10**: 388. 1845.
Stemodia hirsuta Heyne in Wall. Cat. No. 3930. 1831 (nomen), Benth. Scroph. Ind. 24. 1835.

The specimens on which *Columnea chinensis* was based were collected September 27, 1751, on Dane's Island, near Whampoa, and like most of Osbeck's species it has been entirely overlooked by all botanists. The introductory statement, in Swedish, is translated as follows: "Pange-ka, so-called by the Chinese, is an herb which is common along the river, growing mostly in the water along with preceding species [*Cyperus*]. It has a pleasant smell." It is with difficulty placed in any of the known genera as proved by the following description: "*Columnea ? chinensis*: Perianthium duplex: inferius diphyllum, minimum, foliolis subulatis; superius quinquepartitum, laciniis lineari-lanceolatis, tubo brevioribus. Corolla monopetala: Tubus cylindricus; Faux barbata; Limbus quinquelobus, Lobis ovatis; Lucisura inter 2 lobos unius lateris minus profunda, ad quam pistillum & stamina sese vertunt & barba circumdantur. Filamenta 4 filiformia, didynamiae, 2 & 2 adhaerentia; Antherae incumbentes, parvae.

Germen ovatum. Stylus filiformis. Stigma subcapitatum, deflexum. Capsula ovata, polysperma. Herba caule procumbente, tereti, crasso, carnoso, villoso. Folia opposita, oblonga, serrata. Flores ex alis & terminales, caerulei; pedunculi villosi. Habitat ad ripam fluminis."

Melia parasitica Osbeck, Dagbok Ostind. Resa 278. 1757 = *Lansium domesticum* Jack.

The introductory statement in Swedish, regarding this species, translates as follows: "A small herb of barely a finger's length growing on the tree trunks. It is so rare that, so far as is known, no one ever saw it before." Following this is the description in Latin: "Perianthium monophyllum, tridentatum, cylindricum, corolla dimidio brevius. Corolla monopetala, cylindrica, 5 partita; petalis oblongis. Nectarium campanulatum, margine praemorsum, cujus margini interiori filamenta 10, brevissima vix conspicua, inserta. Antherae subquadratae. Germen cylindricum, pentagonum; stylus subulatus, basi lanatus; stigma capitatum. Flores racemosi. Folia nulla."

The description calls for a plant with a peculiar combination of characters, a small, epiphytic or parasitic, leafless herb with meliaceous flowers. There is no plant in existence presenting the above characters, yet Osbeck's description is susceptible of explanation. He undoubtedly saw the characteristic cauliflorous inflorescence of *Lansium domesticum* Jack, a tree in common cultivation throughout Java, and cauliflory being a phenomenon quite new to him, he explained it by assuming the racemose inflorescence, growing directly from the trunk of the tree, to be a leafless parasitic plant. However, Osbeck's description does not apply entirely to *Lansium* flowers, as he describes the calyx as 3-toothed, the staminal tube as campanulate, and the style as subulate. It is possible that he saw a cauliflorous species of *Dysoxylum*, but all the known Javan cauliflorous species of this genus are described as having 4-merous, not 5-merous flowers. Osbeck's specimens were collected at New Bay, western Java, January 20, 1752.

Monarda chinensis Osbeck, Dagbok Ostind. Resa 240. 1757 = ?

This was from Dane's Island, near Whampoa, China, and was observed by Osbeck October 20, 1751. The original description, introduced by a statement in Swedish to the effect that the species grew on bare hills, is as follows: "*Monarda chinensis.* Perianthium duplex; superius 5 phyllum foliolis linearibus, inferius diphyllum. Corolla

monopetala; tubus cylindricus, calyce longior; Limbus bilabiatus, Labium superius integrum, minimum, inferius trilobum deflexum, longius. Stamina duo. Pistillum unicum. Flores minimi, lutei, ex alis. Caulis fibrosis. Habitat in aridis locis."

If this species be a labiate, then no representative of the entire family at present known from the Province of Kwangtung agrees entirely with Osbeck's description. Possible identifications are *Salvia plebeia* R. Br. and *Mosla lanceolata* Max., but Osbeck's description agrees with neither. The only other genera of *Labiatae* known from Kwangtung that have but two stamens are *Lycopus* and *Salvia*, and assuredly *Monarda chinensis* Osbeck can be referred to neither of these. It is possible that Osbeck's description applies to some representative of the *Acanthaceae* or the *Scrophulariaceae* in genera having flowers with but two stamens, and again it is possible that the description was in part based on erroneous observations. It is certainly no *Monarda*.

Phytolacca ? javanica Osbeck, Dagbok Ostind. Resa 276. 1757 = *Terminalia catappa* L.

Osbeck's description and specific name antedates that of Linnaeus, but fortunately the Linnæan name *Terminalia catappa* is saved by the fact that Miquel has described a different species as *Terminalia javanica*. *Phytolacca* ? *javanica* was observed near Anjer, Java, January 20, 1752. The associated plants mentioned by him are *Sophora alopecuroides* (probably an erroneous identification for *S. tomentosa*), *Morinda citrifolia*, *Guettarda speciosa*, *Lobelia plumierii* = *Scaevola*, *Crinum asiaticum*, *Corypha umbraculifera*, *Cordia myxa*, *Flagellaria indica*, and *Convolvulus pes-caprae*, a typical Malayan strand association. The full description, introduced by a short statement in Swedish to the effect that it was a large tree standing on the seashore, with smooth leaves and downy branchlets, is as follows: "*Phytolacca ? javanica*. Perianthium nullum. Corolla monopetala, 5 fida; Lobis ovatis, minimis. Filamenta decem filiformia, superne curva, corollae tubo inserta, corolla longiora. Antherae subrotundae. Arbor ramosissima. Petiola & rami tomentosa. Folia lato-lanceolata, petiolata, integerrima, glabra, axillaria, 7 nervia. Flores parvi, racemosi." There are slight discrepancies in the description, as, for example, the leaves described as "lato-lanceolata"; in the normal form of *Terminalia catappa* the leaves are obovate, but are subject to considerable variation. No other Indo-Malayan strand tree at all agrees with Osbeck's description.

Rhamnus thea Osbeck, Dagbok Ostind. Resa 161, 232. 1757 = *Sageretia theezans* (L.) Brongn.

This is listed in Index Kewensis as "*Rhamnus thea* Osb. It. 232 = Sageretia theezans." Osbeck's name is cited by Linnaeus as a synonym in the original description of *Rhamnus theezans* L. Mant. **2**: 207. 1771. However, while there is no doubt as to the identity of *Rhamnus thea*, and this name is older than *Rhamnus theezans* L., I do not consider that *Rhamnus thea* is properly published by Osbeck, and hence do not accept his specific name. There is no Latin description, and a translation of Osbeck's remarks is as follows: "*Rhamnus thea*, or poor man's tea, is a bush about 6 feet high, the leaves of which resemble those of our common tea, but the flowers belong to the first order of the fifth class [*Pentagynia, Monandria*], are quite small, spicate, on the much branched branchlets. It is used by the poor as tea and here served as a fence. It is called by the Chinese *Tja*.

Scirpus chinensis Osbeck, Dagbok Ostind. Resa 220. 1753.

Scirpus squarrosus L. Mant. **1**: 181. 1767.

The original description of this species is as follows: "*Scirpus* (*chinensis*) culmo triquetro, subnudo, spicis ternis, sessilibus, terminalibus, involucro diphyllo, reflexo." In Swedish follows a statement to the effect that it is a grass with long narrow leaves, one of those subtending the spike being much longer than the other, and with a reference to *Motta pullu* Rheede, Hort. Malabar. **12**: 71. *pl. 38* as representing the species.

Scirpus chinensis Osbeck is listed in Index Kewensis, the original publication is correctly cited, as to page, but not as to title: "*Scirpus chinensis* Osb. It. 220," and it is reduced to *Scirpus squarrosus* L., to which Linnaeus also referred Rheede's figure. There is practically no doubt as to the correctness of this identification of Osbeck's species, but his name is much older than that of Linnaeus and it is here adopted. Osbeck's specimen was collected September 23, 1751, on "Frans" (= French) Island, near Whampoa. Dunn & Tutcher[12] cite the species from Whampoa.

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[12] Fl. Kwangtung and Hongkong 301. 1912.

Solidago chinensis Osbeck, Dagbok Ostind. Resa 241. 1759 = ?

This was from Dane's Island, near Whampoa, October 20, 1751, and certainly is no *Solidago*. *Solidago virgaurea* L. occurs in Kwangtung Province, but not at low altitudes. The original description, which is entirely inadequate, is as follows: "*Solidago* (*chinensis*) caule procumbente, ramis alternis, foliis radicalibus linearibus. Obs. vix pedalis." The description is rather definite, and it is possible that the identity of the species may be determined at a later date. The name does not appear in Index Kewensis.

Tetradapa javanorum Osbeck, Dagbok Ostind. Resa 93. 1757 = *Erythrina indica* Lam.

The original description of this species is as follows: "*Tetradapa Javanorum. Erythrina Corallodendron* ? Descr. Perianthium monophyllum, spathaceum, breve, ovatum. Vexillum magnum, includens petala 4 ovalia, breviora. Stamina decem, quorum 9 ad medium connexa. Filamenta subulata. Antherae erectae oblongae. Germen longum, lanatum. Stylus subulatus. Stigma pubescens, nutans. Flores racemosi sive verticillati, rubri, caduci. Fructus, sub arbore jacens (si hujus arboris non dicam) erat legumen rhomboideum compressum. Semina duo reniformia." The description is followed by a statement in Swedish regarding the size of the tree, color of its flowers, etc., and a reference to *Gelala litorea* Rumph. Herb. Amb. **3**: 231. *t.* 77, which is *Erythrina indica* Lam. The specimens were observed by Osbeck near Anjer, Java, July 16, 1751, associated with numerous other typical strand plants.

It is apparent that Osbeck intended " Tetradapa " as a new generic name, although it is not so indicated, and both the generic and specific name have been overlooked by all subsequent botanists; *Tetradapa* is no Javanese name. While *Tetradapa javanorum* Osbeck is unquestionably an exact synonym of *Erythrina indica* Lam., it seems to be necessary to adopt, as the oldest specific name for the species, *Erythrina variegata* L. in Stickman Herb. Amb. 10. 1754, Amoen. Acad. **4**: 122. 1759, which is based solely on *Gelala alba* Rumph. Herb. Amb. **2**: 234. *pl.* 77. *Gelala alba* Rumph. = *Erythrina variegata* L. is merely a form of the common *Erythrina indica* Lam. with slightly variegated leaves, and presents no characters by which two species may be distinguished. Unless this name be accepted, the rules of priority would necessitate the transfer of *Tetradapa javanorum* to *Erythrina*, for

Erythrina indica Lam. is antedated by both *E. variegata* L. and by Osbeck's name. The synonymy is as follows:

Erythrina variegata L. in Stickman Herb. Amb. 10. 1754; Amoen. Acad. **4**: 122. 1759.

Gelala alba Rumph. Herb. Amb. **2**: 234. *pl. 77.* 1750.
Erythrina corallodendron var. *orientalis* L. Sp. Pl. 706. 1753.
Tetradapa javanorum Osbeck, Dagbok Ostind. Resa 93. 1757.
Erythrina picta L. Sp. Pl. ed. 2. 993. 1763, saltem quoad *Gelala alba* Rumph.
Erythrina indica Lam. Encycl. **2**: 391. 1785.
Erythrina orientalis Murr. Comm. Gotting. **8**: 35. *pl. 1.* 1787.
Erythrina lithosperma Blume, Cat. Gew. Buitenz. 92. 1823.
Erythrina carnea Blanco, Fl. Filip. 564. 1837.

The form with slightly variegated leaves, well figured by Rumphius, is *Erythrina variegata* L.; the form with the leaves not variegated is *Erythrina indica* Lam. *Erythrina variegata* is cultivated in Amboina (*Rumphius*), in Mindanao (*Merrill*), in Palawan (*Curran*), and doubtless in various other islands in the Malay Archipelago and the Philippines, and is typical *Erythrina indica* Lam. in all respects except in the slight variegation of its leaves. A parallel case is presented by the two prominent forms of *Graptophyllum pictum* (L.) Griff., as to leaf color, but which no botanist now considers represent other than a single species.

Torenia glabra Osbeck, Dagbok Ostind. Resa 210. 1757.

Torenia benthamiana Hance, Ann. Sci. Nat. IV. Bot. **18**: 226. 1862.

Osbeck's name is much the older and should be retained for this species; it does not appear in Index Kewensis. The original description is as follows: "*Torenia glabra*. Descr. Perianthium 5 angulare, erectum, laciniis 5 lineari-lanceolatis, tubo brevioribus. Corolla ringens; Labium superius subintegrum, reflexum; Labium inferius trilobum, deflexum. Stamina 4, corolla breviora, quorum 2 tubo breviora, per paria connexa, labio inferiori imposita; superiora altero ramo sterili. Stylus filiformis; stigma cochleatum, bifidum. Capsula longa, unilocularis? Semina plurima. Flores ex alis. Folia ovata, crenata, subsessilia, opposita. Habitat etjam inter agros Oryzae insulae Danicae."

The specimens on which the description was based were from

Dane's Island, near Whampoa, September 10, 1751. *Torenia benthamiana* Hance, which is certainly a synonym of *T. glabra* Osbeck, is reported by Dunn & Tutcher[13] from Hongkong, Swatow, and Whampoa.

Trapa bicornis Osbeck, Dagbok Ostind. Resa 191. 1757.

This appears as a footnote under the Chinese name *ling-kamm* or *leng-ka*, as "*Trapa bicornis* vid. Plum. Icon. T. 67," with a statement in Swedish to the effect that the fruit looks like two horns joined together, with a kernel in the middle, that it is sold in the shops, and that it is eaten by poor people. Osbeck's name antedates that of the younger Linnaeus who is generally cited as the authority for the species.[14] *Trapa bicornis* is, by many authors, considered only a form of *Trapa natans* L.

Urena chinensis Osbeck, Dagbok Ostind. Resa 225. 1757 = ? *Urena lobata* L.

This is listed in Index Kewensis as follows: "*Urena chinensis* Osbeck, Voy. ed. Angl. 1, 363 (Quid?)," the English edition of Osbeck's work having been published in the year 1771. The description is inadequate, but the plant is probably merely a form of *Urena lobata* L. The original description is as follows: "*Urena* (*chinensis*) caule erecto, floribus majoribus," followed by a statement in Swedish to the effect that it grew on the lower slopes of hills not far from the shore. It was observed near Whampoa, September 22, 1751.

Additions to Index Kewensis

Achyranthes chinensis Osbeck, Dagbok Ostind. Resa 205. 1757 = ? *Achyranthes indica* L.

Briza elegans Osbeck, Dagbok Ostind. Resa 246. 1757 = ? *Eragrostis elegantula* Steud.

Caryota javanica Osbeck, Dagbok Ostind. Resa 270. 1757 = *Ceratolobus javanicus* Merr. (*C. glaucescens* Blume).

Catesbaea ? **javanica** Osbeck, Dagbok Ostind. Resa 92. 1757 = *Clerodendron commersonii* Spreng.

Citrus grandis Osbeck, Dagbok Ostind. Resa 98. 1757 (*C. decumana* L.).

Citrus limonia Osbeck, Reise Ostind. China 250. 1765.

Citrus sinensis Osbeck, Dagbok Ostind. Resa 41. 1757, *nomen*, Reise Ostind. China 250. 1765.

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[13] Fl. Kwangtung and Hongkong 188. 1912.

[14] L. f. Suppl. 128. 1781.

Columnea chinensis Osbeck, Dagbok Ostind. Resa 230. 1757 = *Limnophila chinensis* Merr. (*L. hirsuta* Benth.).

Commelina chinensis Osbeck, Dagbok Ostind. Resa 242. 1757 = *C. nudiflora* L.

Cryptanthus chinensis Osbeck, Dagbok Ostind. Resa 215. 1757 = ?

Melia parasitica Osbeck, Dagbok Ostind. Resa 277. 1757 = *Lansium* vel *Dysoxylum*.

Mimosa chinensis Osbeck, Dagbok Ostind. Resa 233. 1757 = *Albizzia chinensis* Merr. (*A. stipulata* Boiv.).

Monarda chinensis Osbeck, Dagbok Ostind. Resa 240. 1757 = ?

Phytolacca ? **javanica** Osbeck, Dagbok Ostind. Resa 276. 1757 = *Terminalia catappa* L.

Solidago chinensis Osbeck, Dagbok Ostind. Resa 241. 1757 = ?

Tetradapa javanorum Osbeck, Dagbok Ostind. Resa 93. 1757 = *Erythrina indica* Lam. = *E. variegata* L.

Torenia glabra Osbeck, Dagbok Ostind. Resa 210. 1757 (*T. benthamiana* Hance.)

CORRECTIONS TO INDEX KEWENSIS

Clematis chinensis Osbeck, Dagbok Ostind. Resa 205, 242. 1757, in place of "Osb. iter. ed. Angl. 1: 393" [1771].

Hedyotis herbacea Osbeck, Dagbok Ostind. Resa 245. 1757, in place of "Osbeck Voy. ed. Angl. 4" [1771] = *Oldenlandia corymbosa* L.

Hedysarum styracifolium Osbeck, Dagbok Ostind. Resa 247. 1757, in place of "*Hedysarum styracifolium* L. Syst. ed. 10. 1169 [1759] = *Desmodium styracifolium* Merr.

Holcus latifolius Osbeck, Dagbok Ostind. Resa 247. 1757, in place of "*Holcus latifolius* L. Syst. ed. 10. 1305" [1759] = *Centotheca latifolia* (Osbeck) Trin.

Hypericum chinense Osbeck, Dagbok Ostind. Resa 244. 1757, in place of "*Hypericum chinense* L. Syst. ed. 10, 2, 1184" [1759].

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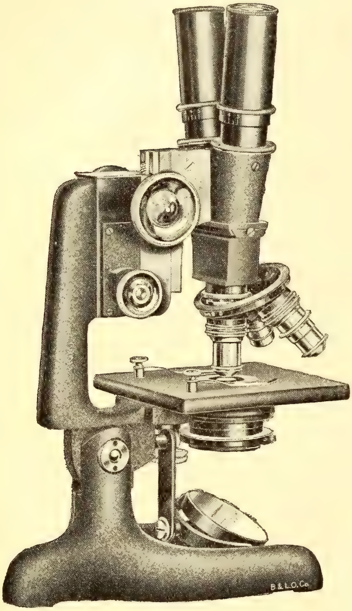

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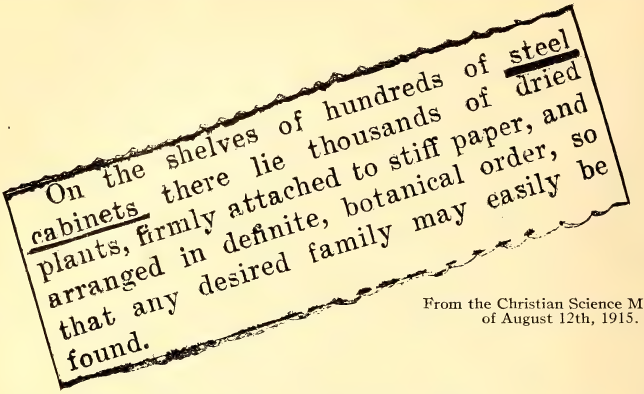

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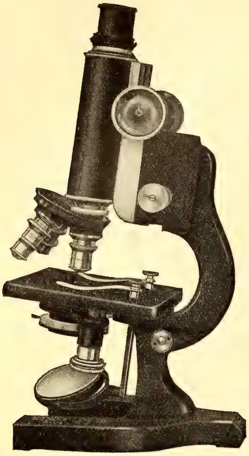

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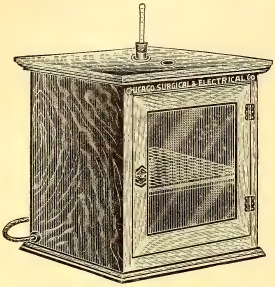

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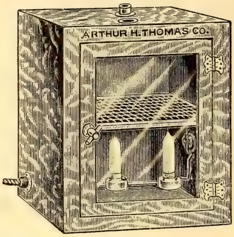
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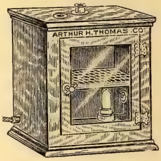
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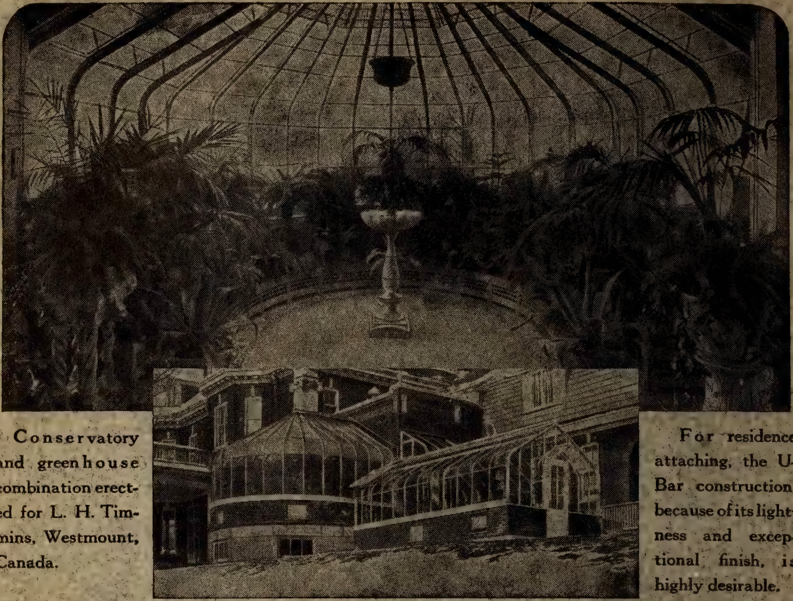

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